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THE UNIVERSITY OF ALBERTA

EFFECTS OF THE CARBAMATE INSECTICIDE, CARBOFURAN, ON MACROINVERTEBRATES IN PRAIRIE PONDS

by

MARK WAYLAND

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA
SPRING, 1989

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled EFFECTS OF THE CARBAMATE INSECTICIDE, CARBOFURAN, ON MACROINVERTEBRATES IN PRAIRIE PONDS submitted by MARK WAYLAND in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

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Supervisor

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Date..... December 8, 1995

Abstract

This study focuses on the effects of the carbamate insecticide, carbofuran, which is used extensively for agriculture on the Canadian prairies, on macroinvertebrates in prairie ponds. It was prompted by the lack of information about the fate and effects of this insecticide in aquatic ecosystems as well as the recent concern expressed by wildlife biologists over the possible deleterious effects of drift and direct overspraying, during aerial applications of insecticides, on the food webs within prairie potholes, particularly the macroinvertebrate component which is an important constituent in the diets of several species of prairie ducks.

There were four components to the study: 1) a multi-pond field study conducted during 1986 in which the abundances of several macroinvertebrate taxa were recorded on several dates before and after a mid-July application of carbofuran that yielded concentrations in the water column of the treatment ponds of ca. 9-32 µg/L, 16 h after spraying; 2) an enclosure study conducted in a single pond during 1987 in which the abundance and biomass of several taxa were recorded in each of 21 enclosures twice before and four times after a mid-July application of carbofuran at three treatment levels (0, 5, and 25 µg/L); 3) a cage study conducted during 1986 and 1987 which permitted the assessment of the *in-situ* toxicity of carbofuran to selected macroinvertebrates; and 4) a laboratory study which examined the influence of pH on carbofuran-induced mortality to *Hyalella azteca*.

In 1986, the fate of carbofuran was also examined in the four treatment ponds. Carbofuran degraded rapidly with half-lives in the four ponds ranging from 30-74 h in the water column and 48-58 h in submersed macrophytes. Partitioning of carbofuran was highly variable among and, especially, within ponds (CV=71%); mean concentrations ranged from 3-46 times greater in aquatic plants than in the water column up to 124 h after spraying. Carbofuran did not appear to accumulate in the sediments.

Among the aquatic macroinvertebrates considered in this study, the amphipods *Hyalella azteca* and *Gammarus lacustris*, large midge larvae of the genus *Chironomus*, particularly *C.*

tentans, and caddisfly larvae, particularly those of the genus *Limnephilus* were susceptible to carbofuran in the range of 9-32 µg/L, but appeared to be resistant at 5µg/L. Damselfly nymphs, particularly *Enallagma*, and small Chironominae larvae, particularly *Tanytarsus*, *Paratanytarsus*,and *Glyptotendipes*, did not appear to be reduced by carbofuran in the range of 9-32 µg/L. Tanypodinae larvae, two snail genera (*Physa* and *Helisoma*) and the leech, *Helobdella stagnalis* did not appear to be affected by carbofuran up to 25 µg/L.

Recovery of *H. azteca* following its initial carbofuran-induced decline was not apparent in the multi-pond study but was apparent in the enclosure study. Possible reasons for this difference are discussed. In 1986, the abundance of trichopteran larvae recovered within 1 month of the mid-summer application. Secondary effects of carbofuran on macroinvertebrates during the post-treatment period were not overt. However, it appeared that ecological release from competition with *H. azteca*, *Chironomus*, or both, may have permitted the abundance and biomass of *Helisoma* to remain high throughout the post-treatment period in the 25 mg/L enclosures, a pattern which was not evident in the controls. Finally, pH influenced mortality rates of *H. azteca* when exposed to various concentrations of carbofuran. Estimated LC50 values were ca. 9, 13, and 21 µg/L at pH's approximating 7, 8, and 9 respectively.

Some implications of carbofuran use on the prairies for breeding ducks and their broods are discussed.

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Table of Contents

Chapter	Page
I. INTRODUCTION	1
LITERATURE CITED	2
II. FATE OF CARBOFURAN AND ITS EFFECTS ON AQUATIC MACROINVERTEBRATES IN PRAIRIE PONDS: A MULTI-POND APPROACH	5
INTRODUCTION	5
Fate and persistence	5
Effects on macroinvertebrates	5
STUDY AREA	6
METHODS	7
Fate and persistence	7
Effects on macroinvertebrates	10
RESULTS	12
Fate and persistence	12
Effects on macroinvertebrates	17
DISCUSSION	31
Fate and persistence	31
Effects on macroinvertebrates	33
LITERATURE CITED	38
III. EFFECTS OF CARBOFURAN ON MACROINVERTEBRATES IN A PRAIRIE POND: AN ENCLOSURE APPROACH	47
INTRODUCTION	47
STUDY AREA	48
METHODS	48
RESULTS	52
DISCUSSION	68
LITERATURE CITED	80
IV. TOXICITY OF CARBOFURAN TO SELECTED MACROINVERTEBRATES IN PRAIRIE PONDS	87
INTRODUCTION	87
STUDY AREA AND METHODS	88
1986	88
1987	89

RESULTS 90
1986 90
1987 94
DISCUSSION 94
General 94
1986 96
1987 101
LITERATURE CITED 102
V. THE INFLUENCE OF pH ON MORTALITY RATES OF THE AMPHIPOD, *Hyalella azteca*, EXPOSED TO CARBOFURAN 107
INTRODUCTION 107
METHODS 107
RESULTS 108
DISCUSSION 109
LITERATURE CITED 114
VI. CONCLUSION 117
EFFECTS ON MACROINVERTEBRATES 117
POTENTIAL EFFECTS ON PRAIRIE-PARKLAND WATERFOWL 118
LITERATURE CITED 128
APPENDIX I 133
APPENDIX II 140
APPENDIX III 143
APPENDIX IV 144

List of Tables

Table		Page
II-1	Selected physico-chemical characteristics of the study ponds.	8
II-2.	Concentrations of carbofuran in the water column, submersed plants, and sediments in the four treatment ponds at two times after spraying.	13
II-3	Rates of loss of carbofuran from water and submersed plants in four ponds sprayed with this insecticide.	14
II-4	Bioconcentration factors (mean±1SE, n=4) of carbofuran in aquatic plants in the treatment ponds.	16
III-1	Some physico-chemical features of water within enclosures based on samples taken after the treatment date (July 15).	49
III-2	Temperature, pH, and concentrations of carbofuran in the water column 2 h after treatment.	53
III-3	Summary of repeated measures ANOVA on various taxa of macrobenthos.	56
III-4	Percent of *Chironomus* larvae still alive in dip net samples taken 48-72 h after treatment.	61
IV-1	Analysis of the effect of carbofuran on selected taxa of macroinvertebrates held in cages at different depths in 1986.	92
IV-2	Percentage of animals surviving or found dead after 72-96 h of exposure in cages in shallow and deep zones of study ponds.	93
IV-3	Percentage of animals surviving or found dead in cages 72-96 h after enclosures were treated with carbofuran in 1987.	95

V-1 The pH of water in test beakers used in the 96-h test of the toxicity of carbofuran to *Hyalella azteca*. 110
V-2 Percentage mortality of *Hyalella azteca* exposed to various concentrations of carbofuran at three pH levels. 111
V-3 Estimated 96-h LC50's and relative toxicities of carbofuran to *Hyalella azteca* at three pH levels. 112
VI-1 Summary of the invertebrate component in the diets of breeding prairie ducks. 121
VI-2. Summary of the invertebrate component in the diets of ducklings of species of prairie ducks. 125

List of Figures

Figure		Page
II-1	Abundance (mean±SE) of *Hyalella azteca* in shallow and deep zones of two control and two carbofuran-treated ponds 1 and 2 days before and 2 and 3 days after spraying.	18
II-2	Abundance (mean±SE) of Chironominae larvae in two control and two carbofuran-treated ponds 1 and 2 days before and 2 and 3 days after spraying.	20
II-3	Abundance (mean±SE) of coenagrionid nymphs in two control and two carbofuran-treated ponds 1 and 2 days before and 2 and 3 days after spraying.	21
II-4	Abundance (mean±SE) of trichopteran larvae in two control and two carbofuran-treated ponds 1 and 2 days before and 2 and 3 days after spraying.	23
II-5	Abundance (mean±SE) of *Hyalella azteca* in shallow and deep zones of four control and four carbofuran-treated ponds from June, 1986 until May, 1987.	24
II-6	Abundance (mean±SE) of coenagrionid nymphs in four control and four carbofuran-treated ponds from June, 1986 until May, 1987.	26
II-7	Abundance (mean±SE) of Chironominae larvae in four control and four carbofuran-treated ponds from June, 1986 until May, 1987.	27
II-8	Abundance (mean±SE) of the mayfly, *Caenis* in shallow and deep zones of four control and four carbofuran-treated ponds from June, 1986 until May, 1987.	28
II-9	Abundance (mean±SE) of trichopteran larvae in four control and four carbofuran-treated ponds from June, 1986 until May, 1987.	29
III-1	Abundance and biomass (mean±SE, n=7) of *Hyalella azteca* in control, 5-µg/L, and 25-µg/L enclosures on 6 sampling dates during summer, 1987.	55

III-2 Abundance and biomass (mean±SE, n=7) of Chironominae larvae in control, 5-µg/L, and 25-µg/L enclosures on 6 sampling dates during summer, 1987. 58
III-3 Abundance (mean±SE, n=7) of *Chironomus* larvae in control, 5-µg/L, and 25-µg/L enclosures on 6 sampling dates during summer, 1987. 60
III-4 Abundance and biomass (mean±SE, n=7) of Tanypodinae larvae in control, 5-µg/L, and 25-µg/L enclosures on 6 sampling dates during summer, 1987. 62
III-5 Abundance and biomass (mean±SE, n=7) of the snail, *Physa*, in control, 5-µg/L, and 25-µg/L enclosures on 6 sampling dates during summer, 1987. 64
III-6 Abundance and biomass (mean±SE, n=7) of the snail, *Helisoma*, in control, 5-µg/L, and 25-µg/L enclosures on 6 sampling dates during summer, 1987. 65
III-7 Abundance and biomass (mean±SE, n=7) of the leech, *Helobdella stagnalis* in control, 5-µg/L, and 25-µg/L enclosures on 6 sampling dates during summer, 1987. 67
III-8 Abundance and biomass (mean±SE, n=7) of coenagrionid nymphs in control, 5-µg/L, and 25-µg/L enclosures on 6 sampling dates during summer, 1987. 69
III-9 Abundance and biomass (mean±SE, n=7) of mayflies of the genus *Caenis* in control, 5-µg/L, and 25-µg/L enclosures on 6 sampling dates during summer, 1987. 70
III-10 Abundance (mean±SE, n=7) of Chironomidae pupae in control, 5-µg/L, and 25-µg/L enclosures on 6 sampling dates during summer, 1987. 75
IV-1 Mean percentage of caged individuals (n=6 cages) of three macroinvertebrate taxa surviving after 72-96 h at shallow and deep sites in each of the four carbofuran-treated and four control ponds. 98
IV-2 Mean percentage of caged individuals (n=6 cages) of three macroinvertebrate taxa found dead after 72-96 h at shallow and deep sites in each of the four carbofuran-treated and four control ponds. 99

CHAPTER I
INTRODUCTION

The past two decades have witnessed a dramatic increase in the use of insecticides for crop protection on the Canadian prairies. While year-to-year variation in insecticide use is high, it has been estimated that an average of 1.0-1.5 million ha. of prairie farmland are sprayed on an annual basis (Sheehan et al. 1987). Moreover, these authors estimate that in years of severe grasshopper (Orthoptera, Acrididae) outbreaks, the area sprayed rises to 3.0-3.5 million ha.

Aerial application of insecticides has also become more popular in recent years accounting for 8-15% of the total area sprayed in any given year (King 1978, Sheehan et al. 1987).

Because of the extensive use of insecticides and the increasing popularity of aerial application, concern has been expressed that the innumerable small water bodies commonly referred to as prairie potholes which dot the prairie agricultural landscape may become contaminated by either direct overflight spraying or drift deposit from spraying slough borders (Grue et al.1986, Sheehan et al. 1987).

A large proportion of North America's waterfowl relies on prairie potholes as a source of high-protein invertebrate food throughout the breeding and brood-rearing portions of their annual cycle (Swanson & Meyer 1973, Bellrose 1980). Accidental contamination could potentially reduce their invertebrate standing crops and thus seriously degrade their value to waterfowl.

In recent years, the carbamate insecticide carbofuran (2,3-dihydro-2-2 dimethyl-7-benzofuranyl methylcarbamate) has been one of the most widely used insecticides on the Canadian prairies, especially for grasshopper control. However, aside from the well-known relationship between pH and hydrolysis of carbamate insecticides (Aly & El-Dib 1972, Charnetski et al. 1977, Chapman & Cole 1982) and the work done on the persistence of granular carbofuran in tropical rice paddies (Seiber et al. 1978, Siddaramappa et al. 1978) and of technical-grade carbofuran in a model ecosystem (Yu et al. 1974) the fate of carbofuran in aquatic systems remains poorly understood (NRCC 1979).

Even less is known about the effects of carbofuran on aquatic macroinvertebrates. The sum of our information on this topic

appears limited to laboratory or field studies of its acute toxicity to *Daphnia* (Yu et al. 1974, Hartman & Martin 1985, Johnson 1986), midge larvae (Diptera: Chironomidae) (Mulla and Khasawinah 1969, Karnak & Collins 1974, Johnson 1986) and mosquito larvae (Mulla et al. 1966, Lancaster & Tugwell 1969).

In this thesis, I present in Chapter II the results of a multi-pond study, the objectives of which were to describe the fate and persistence of carbofuran in prairie ponds and to evaluate its immediate and seasonal effects on the abundances of selected groups of macroinvertebrates. Chapter III deals with an enclosure experiment within a single pond, the objectives of which were to explore in greater depth the response of pond macroinvertebrate assemblages to carbofuran contamination under more controlled field conditions. Chapter IV examines the results of *in-situ* cage experiments which were used to assess the acute effects of carbofuran on selected groups of macroinvertebrates known for their importance as waterfowl foods. Because carbofuran degradation rates are known to vary with pH, and since the pH of prairie wetlands is known to range from near neutrality to greater than 9.0 (Wright 1968, Driver & Peden 1977), I conducted a laboratory test which was designed to evaluate the effects of pH on the acute toxicity of carbofuran to the amphipod, *Hyalella azteca*. This experiment is discussed in Chapter V.

Chapter VI integrates the results of the previous chapters and reviews the waterfowl food habits literature in an attempt to answer the question: could carbofuran contamination of prairie potholes have a deleterious effect on their value as feeding sites for breeding waterfowl and their broods?

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CHAPTER II

FATE OF CARBOFURAN AND ITS EFFECTS ON AQUATIC MACROINVERTEBRATES IN PRAIRIE PONDS: A MULTI-POND APPROACH

INTRODUCTION.

Fate and persistence: Bioavailability of pesticides to aquatic organisms is strongly influenced by factors affecting their fate and persistence in the water (Nimmo 1985, Spacie & Hamelink 1985). In order to evaluate the potential toxicity of a pesticide in a particular environment, it is essential to understand to what extent the chemical is likely to be degraded or adsorbed to particulate matter in that environment. Laboratory studies on the fate of carbofuran in aquatic systems have demonstrated that it is highly susceptible to hydrolysis (Charnetski et al. 1977, Chapman & Cole 1982). Field studies indicate very rapid loss of carbofuran from alkaline pond water (Charnetski et al. 1977, Seiber et al. 1978, Siddaramappa et al. 1978). The high water solubility of carbofuran (415 ppm) coupled with its low octanol-water partition co-efficient (40) (Kenaga & Goring 1980) suggest that sorption to aquatic plants or sediments should be of minor importance in assessing its overall fate in alkaline prairie ponds. Nevertheless, Klaassen & Kadoum (1979) found carbofuran residues in pond sediments 2-8 times higher than in overlying water 3 days after application.

Therefore, in order to understand more fully the potential toxicity of carbofuran in prairie ponds, a preliminary objective of this study was to evaluate its persistence in the water column, and its adsorption to and persistence in submersed aquatic plants and sediments of four prairie ponds treated with surface applications of carbofuran as Furadan 480 Flowable®.

Effects on macroinvertebrates: Relatively, little is known about the effects of carbofuran on aquatic invertebrates. What little information that is available comes, for the most part, from acute single-species toxicity tests done in the laboratory. These studies indicate that carbofuran is a comparatively toxic insecticide with EC50 or LC50 values in the range of 35-48 µg/L for *Daphnia*

(Hartman & Martin 1985, Johnson 1986), 1.6-56.0 ppb for 2 species of *Chironomus* larvae (Karnak & Collins 1974, Johnson 1986) and 54 and 160 ppb for larval *Culex quinquefasciatus* and *Anopheles albimanus* respectively (Mulla et al. 1966b). Enclosure and multi-pond field trials involving carbofuran have been limited to assessing its acute effects on selected macroinvertebrates. These studies indicate that larval populations of mosquitoes and chironomids can be reduced effectively within 24 hr with granular or powdered formulations of carbofuran at approximately 30-90 ppb (Mulla et al. 1966b, Mulla & Khasawinah 1969). However, differences in formulations, application rates and species studied as well as a lack of information about the biotic and physico-chemical nature of the ponds in the above studies make it impossible to interpret their results in terms of the acute effects of carbofuran on macroinvertebrates in prairie ponds.

Assessing seasonal effects of insecticides provides valuable information not only about acute toxicity but also about secondary effects such as density-dependent recovery rates and shifts in community structure through elimination of competitors, prey or predators by a pesticide (Hurlbert 1975). Detection of these types of responses is essential to the understanding of the impacts of a pesticide on macroinvertebrate assemblages in ponds. In the past, multi-pond experiments have been a popular approach to assessing seasonal effects of insecticides on the fauna of ponds (Hurlbert et al.1970, Kennedy and Walsh1970, Kennedy et al. 1970, Macek et al. 1972, Sanders et al. 1981, Crossland 1982, Arthur et al. 1983, Gibbs et al. 1984).

Therefore, because the primary and secondary effects of carbofuran on macroinvertebrates in prairie ponds are poorly known, a multi-pond approach was adopted to examine its immediate and seasonal effects on common pond macroinvertebrates.

STUDY AREA.

The study ponds were located in central Alberta approximately 90 km N of Edmonton near the village of Clyde (54°14' N, 113° 36' W). The eight ponds used in this study were dugouts or borrow pits which had been dug in the late 1950's and early 60's. All ponds were

surrounded by agricultural land which was either in fallow or planted with grasses or cereal crops.

The ponds were steep-sided with maximum depths ranging from approximately 1.7-2.8 m. Emergent vegetation, mainly *Typha latifolia*, was restricted to narrow strips around the perimeters of the ponds. Most ponds supported dense growths of submersed vegetation, mainly *Ceratophyllum demersum*, *Myriophyllum exalbescens*, and *Potamogeton* sp., to a depth of 1.0-1.5 m. Some physico-chemical parameters associated with the study ponds are outlined in Table II-1.

METHODS.

Fate and Persistence: Four ponds were randomly assigned for treatment with carbofuran. Since all ponds were rectangular, it was relatively easy to estimate their surface areas. Average pond depths were estimated by recording the depth of each pond at 3-m intervals along both the length and width axes and then averaging these measurements. Estimates of surface area and average depth were used to calculate the volume of each treatment pond on the day it was sprayed.

Two ponds were sprayed on July 23, the other two on July 30, 1986. The ponds were sprayed from a canoe using a backpack sprayer. Care was taken to ensure uniform application of the insecticide. The target initial concentration in each pond was 14 µg/L, a concentration that might be expected in a pond with a mean depth of 1 m following an accidental contamination from a direct overflight while spraying for grasshoppers at the recommended rate.

Sixteen hours after spraying, water, submersed plant and 5-cm thick sediment samples were taken at two shallow sites (25-75 cm) and two deep sites (76-125 cm) within each pond. Water samples were taken 15-30 cm below the surface and stored in 2-L glass jars. H_2SO_4 was added immediately to lower the pH to 5.0-6.0. Sediment samples were obtained with a 5-cm diameter core sampler. Water temperature and pH were also recorded at each site. All samples were stored on ice in plastic coolers and returned to the laboratory within 8 h. This procedure was repeated 124 h after spraying.

In the laboratory, sediment and plant samples were frozen for

Table II-1. Selected physico-chemical characteristics of the study ponds.

Pond#	pH[1]	Conductivity[2] (µmhos/cm)	Turbidity[2] (NTU)	Sediment[3] %O.M.	Dissolved O_2[4] (mg/L)
T1	8.4	1686	5.8	6.43±1.31	7.5±1.4
T2	8.8	953	25.0	4.05±0.60	8.8±0.5
T3	8.9	404	6.6	3.52±1.46	7.4±1.0
T4	8.5	427	5.3	4.53±0.82	8.0±1.0
C1	10.1	341	1.3	--	10.8±0.1
C2	10.1	329	3.4	--	12.0±1.9
C3	8.9	744	6.9	--	8.6±0.4
C4	9.8	326	3.3	--	9.6±0.7

1. Mean values based on averages from 3 sampling dates between July and September (2-4 samples taken during mid-day from each pond on each date). pH determined with Fisher Model 119 pH meter.
2. Values based on one sample from each pond taken during August, 1986. Samples taken at mid-point in water column at a site where the water depth was ca. 50-75 cm. NTU=Nephlometric turbidity units. Determined with a Hach model 2100A Turbidimeter. Conductivity determined with a YSI Model 32 Conductance meter.
3. % O. M.=% organic matter of 5-cm thick core samples. Mean±1SE, n=4 samples per pond.
4. Mean±1SE based on averages of samples taken from near the pond bottom during mid-day on 3 dates between July and September (2-4 samples per pond per date). Dissolved oxygen determined according to Carpenter (1965).

analysis at a later date. Water samples were processed immediately.

Residues were extracted from water samples with dichloromethane (DCM) following addition of NaCl. The extract was then concentrated and 1 mL of the concentrate was exchanged to acetonitrile prior to analysis by reverse-phase high pressure liquid chromatography (HPLC) with an ultra-violet detector.

Sediment samples were thawed, air-dried and then ground and sieved. Subsamples weighing 25 g were placed in a polytron homogenizer and residues were then extracted with methanol. The extract was filtered, exchanged with ethyl acetate and concentrated to 2 mL.

Plant samples were thawed and thoroughly homogenized in a food processor. Twenty-five gram samples along with 100-mL ethyl acetate and 150-g anhydrous sodium sulphate were then blended in a stainless steel blender for 5 min. The extract was then filtered, and concentrated to 5 mL.

Final extracts of both sediment and plant samples were analyzed by gas chromatography/mass spectrometry (GC/MS) in the selected ion mode of operation. This technique was better than HPLC with the U-V detector for distinguishing carbofuran from other organics (G. Nelson, Enviro-Test Labs, pers. comm).

Standards of carbofuran were analyzed along with the three types of samples so they could be quantified. Blanks, spikes and duplicates were analyzed on a 10% basis. Recoveries from water, soil and plant samples were 110±11%, 68±8.8% and 108±12% respectively (mean ±CV). Analysis of duplicates indicated reasonable precision for two of the sample types (avg. CV=13% and 14.7% for water and plants respectively). Duplicates were not done for sediment samples because carbofuran residues in most sediment samples were below the detection limit (0.02 µg/g). Detection limits for water samples and plants were 0.5µg/L and 0.02 µg/g respectively.

The rate of loss (K_{ob}) of carbofuran from water and submersed plants was calculated using regression techniques. Half-lives ($t_{1/2}$) in the water columns of each pond were calculated according to Seiber et al. (1978). The proportions of the total variation in partitioning between water and plants which was attributable to pond, depth and sampling site were calculated. Partitioning was

calculated from the ratio of the concentration of carbofuran in plant material to that in the water column at a given site. Statistical analyses follow Sokal and Rohlf (1981).

Effects on macroinvertebrates: To determine carbofuran's immediate effects on pond macroinvertebrates, two control and two treatment ponds were sampled 2 days and 1 day before spraying, and 3 days and 4 days after spraying. Samples were taken from four permanently marked shallow sites (25-75 cm deep) and four deep sites (75-125 cm) which had been randomly selected at the beginning of the study. The sampling technique was modified from one described by Rosenberg (1973). It involved the use of a stovepipe sampler 150 cm high and 25 cm diameter. The stovepipe was pushed into the substrate at each site, the sediments were agitated with a paddle, and 10 successive 1-L subsamples were drawn from the bottom using a stoppered container,10 cm in diameter attached to a wooden lath. Upon lowering the container to the bottom, the stopper was removed and each 1-L subsample was subsequently passed through a 500-µm sieve. This technique was preferred to the more standard grab, corer or epiphyte sampling techniques because it was easier to use, resulted in reduced sorting time and appeared to yield a better representation of the pond fauna than any other single technique (Wayland, unpubl. data). Samples were preserved in 70% ethanol and returned to the laboratory where they were sorted using a sugar flotation technique (Flannagan 1973). Invertebrates were identified to species or genus whenever possible using common keys (Wiggins 1977, Pennak 1978, Merritt & Cummins 1984). Chironomid larvae were identified only to subfamily. Because of difficulties in identifying early instar damselfly nymphs, this group was identified only to family (Coenagrionidae). Zooplankton was not considered in this study.

To evaluate carbofuran's seasonal effects on macroinvertebrates, four control and four treatment ponds were sampled approximately biweekly from mid-June until late September. A final sampling was carried out in mid-May, 1987. The sampling and sorting procedures followed the same protocol outlined above.

In order to avoid problems associated with pseudoreplication (Hurlbert 1984), average abundances of the numerically dominant groups were calculated for both shallow and deep sites in each pond

on each sampling date. Data obtained from the biweekly sampling program were smoothed in a manner modified from Prepas (1984) by pairing successive sampling dates and calculating average abundances for deep and shallow sites for each pair of weeks. Thus, average abundances within shallow and deep zones in each pond from June 12 were paired with their corresponding values from June 26 and new averages calculated. In a similar fashion, July 10 was paired with July 22, August 6 with August 20 and September 9 with September 24.

Because invertebrate abundance varies greatly with depth in ponds and lakes (Ali & Mulla 1976, Mittelbach 1981, Thorp & Diggins 1982, Morris & Boag 1982, Diggins & Thorp 1985), the Wilcoxon signed-rank test (Snedecor & Cochran 1980) was used to determine whether any of the invertebrate groups considered in this study were distributed differently between depths in the study ponds over the whole season. The analyses were based on the average abundance of each taxon within each depth zone of the study ponds for each pair of weeks. Since it seemed possible that the insecticide might influence invertebrate distribution patterns in the treated ponds during the post-treatment phase of the study, data from the treated ponds during this period were excluded from the analyses. If the distribution of a particular taxon was found to vary with depth over the whole season, data from shallow and deep zones were analyzed separately. However, if the distribution of a taxon was not depth-dependent, data from shallow and deep zones were pooled, an average obtained and subsequent analyses were based on average abundances within each pond as a whole.

Data were transformed to stabilize variances (Downing 1979), and homogeneity of variances was tested using the *F*-max test (Sokal & Rohlf, 1981). Within the text, backtransformed means have been reported (Sokal & Rolhf 1981) for those data sets which required transformation before being statistically analyzed. Otherwise, means and standard errors based on original data are reported. Figures show transformed means and standard errors.

Repeated measures ANOVA (Winer 1971, p.519) was used to evaluate the effects of carbofuran on the abundance of individual taxa. The main effect was treatment (control and insecticide-treated) while the subplot effects were sampling date and treatment-sampling date interaction (TXD). When interaction or

date effects approached significance ($P \leq 0.10$), planned mean comparisons between dates within treatment levels were done according to Winer (1971, p.384); the Dunn-Sidak method (Sokal &Rohlf 1981, p.242) was used to adjust the experimentwise error rate. For the experiment that measured immediate effects, only adjacent sampling dates were compared. For the experiment evaluating seasonal effects, comparisons were made among all post-treatment sampling periods and between the two pre-treatment sampling periods as well as between the last pre-treatment and first post-treatment periods.

RESULTS.

Fate and persistence. Concentrations of carbofuran in the water columns of treatment ponds 1 and 2 (T1 & T2) 16 h after spraying approximated the target concentration of 14 µg/L. In treatment ponds 3 and 4 (T3 & T4) concentrations were approximately double the target value (Table II-2).

As expected, carbofuran degraded rapidly in all treatment ponds (Table II-2). The degradation rates of carbofuran and other carbamate insecticides in water have been shown to be log-linear functions of time (Aly & El-Dib 1972, Seiber et al. 1978, Gibbs et al. 1984). Assuming that degradation rates in the treatment ponds in this study also followed a log-linear pattern over time, it was possible to estimate degradation rates for each pond despite the fact they were sampled only twice following spraying. Table II-3 shows the loss rate constants (K_{ob}) and half-lives ($t_{1/2}$) of carbofuran in water and plants of the four ponds. The $t_{1/2}$ in the water column ranged from 30 h in pond T3 to 74 h in pond T4 (Table II-3). A two-way ANOVA revealed a significant pond x sampling time interaction (P=0.0004, 3,23df) with respect to loss rates from the water column. This indicates that the K_{ob} in the water column between 16 and 124 h post-spray was not the same across all ponds. Unplanned comparisons of slopes (Sokal & Rohlf,1981 p.507) indicated that rate constants in the water column were similar among ponds T1, T2 and T4 but that pond T3 differed from the other three ponds (P<0.05). This difference occurred despite the fact that pond T3 was very similar to both ponds T1 and T2 with respect to pH

Table II-2. Concentrations of carbofuran in the water column, submersed plants, and sediments in the four treatment ponds at two times after spraying.

Pond	Hours Post-spray	Pond pH (Mean, n=4)	Pond Temp. (°C)[1]	Carbofuran concentration[2] Water (µg/L)	Plants (µg/g)	Sediments (µg/g)
T1	16	9.3	19 (17-22)	9.2 (9.1-9.3)	0.30 (0.09-0.97)	0.04 (0.03-0.05)
	124	9.0	--	3.1 (1.2-8.0)[3]	0.08 (0.04-0.16)	ND[4]
T2	16	8.9	19 (18-21)	14.4 (11.8-17.6)	0.08 (0.02-0.25)	ND
	124	9.0	--	4.1 (2.4-6.9)	0.07[5]	ND
T3	16	8.9	19 (17-22)	32.5 (12.9-81.9)	0.44 (0.15-1.28)	0.05[5]
	124	8.9	--	2.8 (1.6-4.7)	0.10 (0.05-0.21)	ND
T4	16	8.0	16 (14-20)	32.6 (24.6-43.2)	0.20 (0.04-0.92)	0.13[5]
	124	8.9	--	11.9 (8.0-17.7)	0.04 (0.02-0.10)[3]	ND

1. Mean daily temperature (range), based on max-min temperatures for 24 h period before sampling.
2. Mean (95% CL) where n=4, values backtransformed from ln(Y+1) where Y=carbofuran concentration.
3. n=3. No carbofuran detected in fourth sample (ND). Detection limit=0.5µg/L for water and 0.02µg/g for plants and sediments.
4. n=0. Carbofuran not detected in any samples.
5. n=1. No carbofuran detected in other three samples.

Table II-3. Rates of loss of carbofuran from water and submersed plants in four ponds sprayed with this insecticide.

Pond	Medium	Intercept[1]	Slope[1]	r-squared[2]	$t_{1/2}$[3] (h)
T1	water[4]	2.382	-0.0102	0.87	68
	plants	5.907	-0.0120	0.61	58
T2	water	2.856	-0.0117	0.89	59
	plants[5]	--	--	--	--
T3	water	3.846	-0.0229	0.90	30
	plants	6.304	-0.0138	0.69	50
T4	water	3.634	-0.0093	0.88	74
	plants[4]	5.530	-0.0143	0.58	48

1. Intercept and slope values based on $Log_e(Y+1)$ where n=8, Y=carbofuran in µg/L of water or ppb plant based on samples taken at 16 and 124 h post-spray.
2. Co-efficient of determination.
3. Half-life.
4. n=7.
5. Carbofuran concentration in three of four samples at 124 hr was below detection limits. No attempt was made to estimate slope or half-life.

and water temperature (Table II-2). Pond T4, which had the longest $t_{1/2}$ in the water column also had the lowest overall pH and water temperature. This is consistent with what is known about the degradation of carbamate insecticides in water (Aly & El-Dib 1972).

Rate of carbofuran loss from aquatic plants ranged from -0.0143 ($t_{1/2}$=48 h) in pond T4 to -0.0120 ($t_{1/2}$=58 h) in pond T1 (Table II-3). Rate of loss from plants in each pond loosely paralleled corresponding loss rates in water suggesting that loss from aquatic plants may have been primarily due to desorption. As carbofuran was hydrolyzed in the water column, desorption from plants may have served to re-establish an equilibrium.

With the exception of pond T1 and one sample from the shallowest site in both ponds T3 and T4, carbofuran was not detected in bottom sediments (Table II-2). Sediments of pond T1 contained 0.038±0.010 µg/g carbofuran 16 h after spraying (mean±1SE). Carbofuran was not detected in any substrate samples taken at 124 h.

Partitioning of carbofuran between the water column and aquatic plants in the treated ponds was highly variable (Table II-4). Based on the ratios of the concentration of carbofuran in plants to that in the water column at any given site, partitioning was low in ponds T2 and T4 relative to ponds T1 and T3. This pattern was consistent between sampling periods with the exception of T3 in which partition co-efficients were noticeably greater at 124 h than at 16 h. The variance within each pond indicated that the ratio of carbofuran in plants to that in the water column was site and, in the case of T3, sampling date specific. When spray date was included as a factor, it accounted for 39% of the total variation in partitioning (arcsine transformed data). The remaining variation (61%) was due to different sampling dates and sites within ponds. Neither ponds nor depths within ponds contributed to the total variation. It is possible that the large proportion of the total variance attributable to spray date may, in fact, have been due to inherent differences between the two sets of ponds themselves rather than the dates on which they were sprayed. Therefore date was excluded and the analysis repeated. In this case ponds accounted for 29% of the total variation while the combination of site and sampling date accounted for 71%.

Table II-4. Bioconcentration factors (Mean±1SE, n=4) of carbofuran in aquatic plants [(ng carbofuran / g plant) / (µg carbofuran / L water)]. Samples from 16 and 124 h post-spraying are combined. .

		Depth	
Spray Date	Pond	Shallow	Deep
July 23	T2	3.12±0.45[1]	11.50±2.74[2]
	T4	8.88±1.74[2]	4.35±1.36
July 30	T1	49.33±17.01	38.34±17.39[2]
	T3	41.18±29.68	46.31±28.05

1. n=2. Carbofuran was not detected in two other samples.
2. n=3. Carbofuran was not detected in the fourth sample.

Effects on macroinvertebrates: Sixty-seven taxa were recorded in samples taken from the study ponds. However, most occurred only rarely (in less than 50% of the samples) in any given pond while several were never found in samples from one or more ponds (Appendix I). I selected five of the most common taxa in samples from all study ponds for analysis: immature plus mature individuals of the amphipod *Hyalella azteca* (hereinafter referred to as *Hyalella*), larvae of the subfamily Chironominae (Chironomidae), nymphs of the mayfly genus *Caenis* (Caenidae), nymphs of the damselfly family Coenagrionidae and larvae of the order Trichoptera. Trichopteran larvae were from nine genera and five families. The taxa which I analyzed accounted for 50% of the total number of macroinvertebrates recorded, the remaining 62 taxa accounting for the other 50%.

Of these five taxa, only *Hyalella* and *Caenis* showed different densities in shallow and deep zones of the study ponds (both taxa: Wilcoxon-signed rank tests, $P<0.01$). The three other taxa showed no differences in their depth distributions ($P=0.09$, 0.10 and 0.38 for Chironominae larvae, coenagrionid nymphs and trichopteran larvae respectively).

To identify any immediate, deleterious effect of carbofuran on macroinvertebrates in the treated ponds, it was necessary to determine whether changes in abundance between sampling dates were of either a different magnitude or direction when compared with the control ponds. Moreover, it was necessary to demonstrate that before spraying with carbofuran any changes in abundance of macroinvertebrates recorded in the treated ponds paralleled those in the control ponds from one sampling date to the next.

Within 2 days after the application of carbofuran, the abundance of *Hyalella* declined significantly in the shallow zones of the treated ponds in a manner which was not paralleled in the control ponds ($F=9.65$, 3,6df, $P=0.01$) (Fig.II-1A) while in the deep zones this pattern was not significant ($F=0.69$, 3,6df $P=0.59$), although there was a trend towards a decline in carbofuran-treated ponds. (Fig.II-1B). In the control ponds, the mean number of *Hyalella* per sample remained relatively constant over the four sampling dates in the shallow zones (21.6, 19.2, 21.1, and 17.0, means backtransformed from LOG10(X+1)), as well as in the deep zones (7.6, 7.6, 6.6, and 6.0). By contrast, in the treatment ponds,

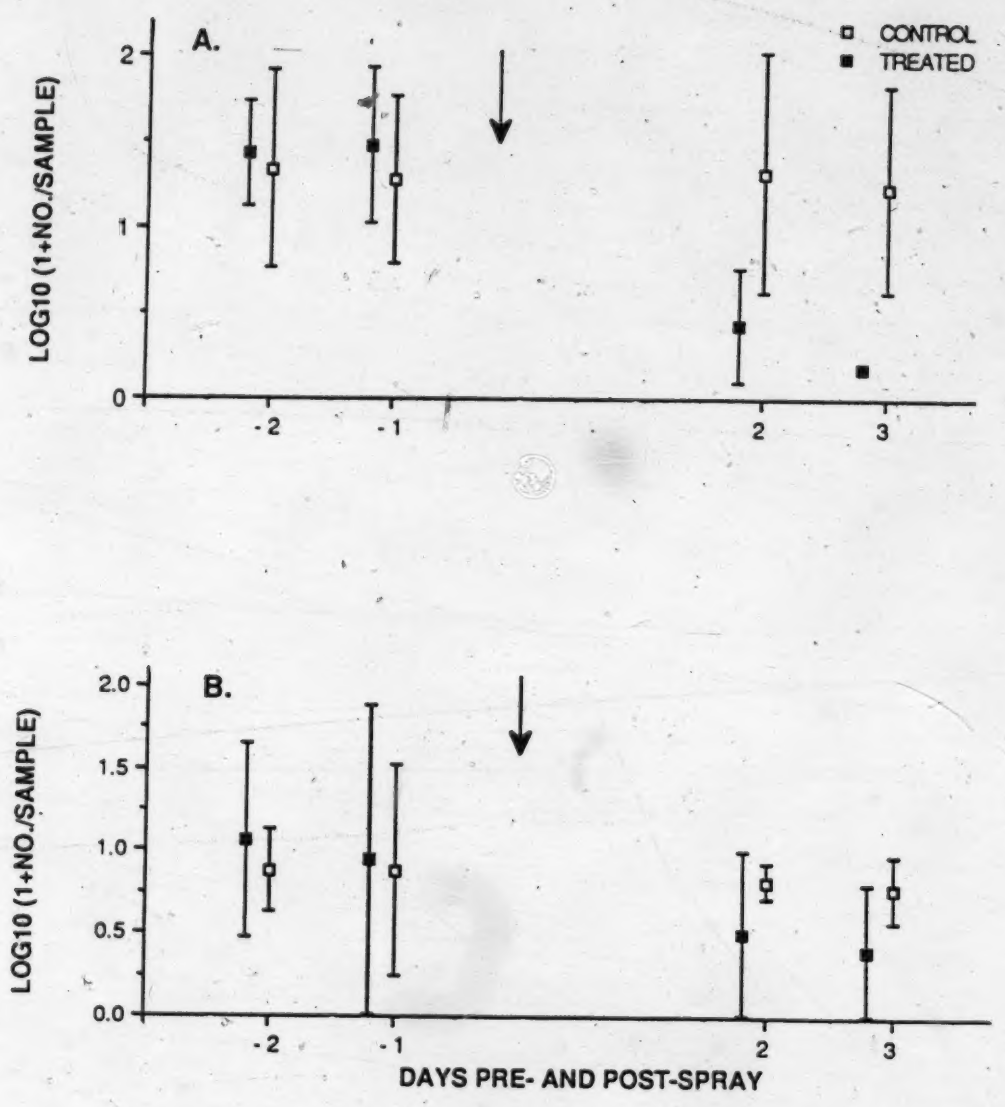

Fig. II-1. Abundance (mean±SE) of *Hyallela* in shallow (A) and deep (B) zones of two control ponds (C2 and 3) and two treated ponds (T2 and 4). Samples were taken 1 and 2 days before spraying and 2 and 3 days after spraying. Carbofuran spraying date (July 23) indicated by arrow. Symbols offset for clarity.

the mean number of *Hyalella* per sample in the shallow zones fell from 26.7 and 30.1 before spraying to 2.7 and 1.5 after spraying; in the deep zones the means were 11.6 and 8.2 before spraying and 3.2 and 2.5 after spraying. In pond T4 alone, *Hyalella* in the deep zone declined from pre-treatment levels of 44.3±5.5 and 77.3±20.2 to post-spray abundances of 9.3±2.7 and 5.5±2.8 (mean±1SE, n=4 samples, based on original data). The only significant change noted, when means of adjacent dates were compared, was in the shallow zones of treated ponds between the last pre-spray and first post-spray sampling dates (t'=5.01, 6df, k=3, P<0.01).

The patterns of change in abundance of larval Chironominae between adjacent dates did not differ significantly in the treatment and control ponds (Fig.II-2) (F=1.33, 3,6df, P=0.35). Thus, spraying with carbofuran did not produce any significant reduction in this taxon.

The analysis of abundance of coenagrionid nymphs showed no statistically significant change (F=1.53, 3,6df, P=0.30) after exposure to carbofuran, based on the experimental procedure used (Fig.II-3) . It is possible, however, that low numbers of nymphs collected in the samples coupled with the high residual variance (CV=79.8%) may have masked any actual reduction in damselfly nymphs following carbofuran application. In pond T2 the mean (±1SE, n=8) numbers of coenagrionid nymphs per sample were 4.3±2.1 and 6.4±3.0, 2 days and 1 day before spraying and 0.5±0.3 and 0.3±0.0, 2 and 3 days after spraying. Similar declines were not noted in pond T4 despite the higher concentration of carbofuran in that pond (Table II-2). The mean (±1SE, n=8) number of coenagrionid nymphs per sample in pond T4 was 4.0±1.5 on July 21 and 10.4±5.2 on July 22. After spraying, their numbers were 8.5±2.0 on July 25 and 4.1±1.7 on July 26. Thus, it is possible that carbofuran reduced coenagrionid abundance in pond T2 but not in pond T4.

Four genera of trichopteran larvae (*Triaenodes, Limnephilus, Molanna*, and *Agrypnia*) occurred in the ponds during late July. The numbers of these four genera were pooled to determine whether or not their abundance was adversely affected by exposure to carbofuran. A significant T X D interaction (F=4.87, 3,6df, P=.048) indicated that abundance of these trichopteran larvae in the treated ponds changed in a manner which was not paralleled in the control ponds. In treated ponds, the only significant change in numbers

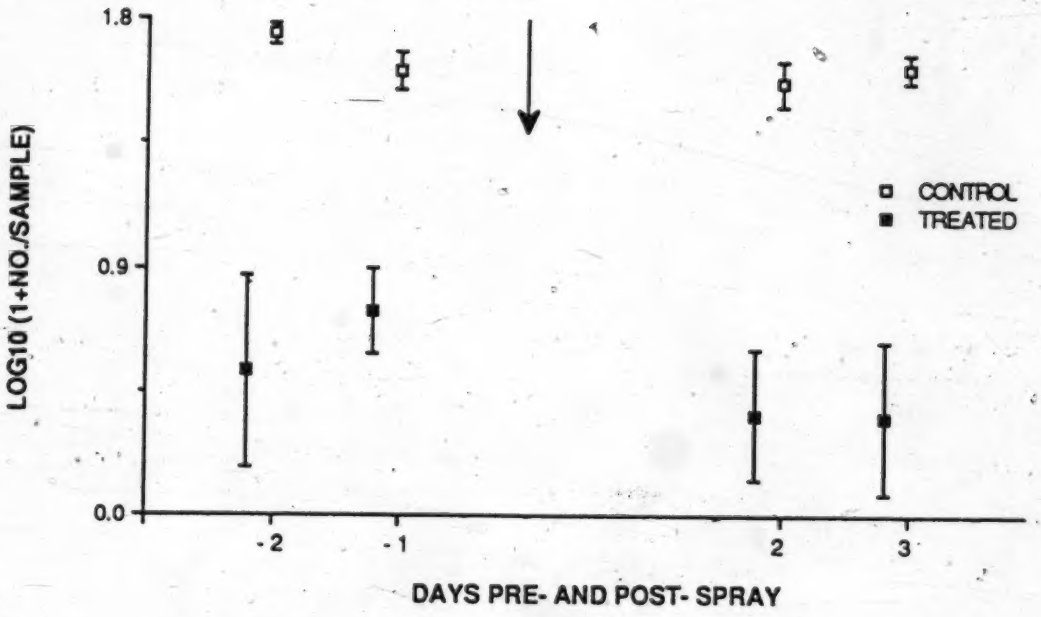

Fig. II-2. Abundance (mean±SE) of Chironominae larvae in two control ponds (C2 and 3) and two treated ponds (T2 and 4). Samples taken 1 and 2 days before spraying and 2 and 3 days after spraying. Carbofuran spraying date (July 23) indicated by arrow. Symbols offset for clarity.

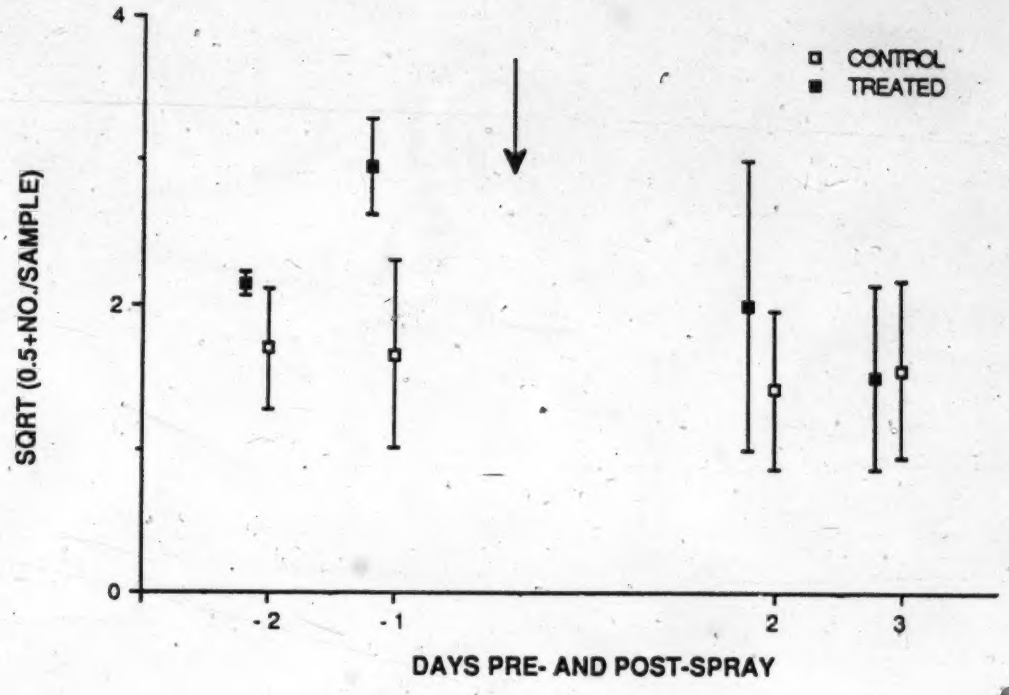

Fig. II-3. Abundance (mean±SE) of coenagrionid nymphs in two control ponds (C2 and 3) and two treatment ponds (T2 and 4). Samples were taken 1 and 2 days before spraying and 2 and 3 days after spraying. Carbofuran spraying date (July 23) indicated by arrow. Symbols offset for clarity.

sampled was between the last pre-spray and the first post-spray dates (t'=3.67, 6df, k=3, P=0.04). Their mean abundance (backtransformed from SQRT(x+0.5)) declined from 1.3 individuals per sample on the last pre-spray sampling date to 0.1 individuals per sample on the first post-spray sampling date (Fig.II-4). In control ponds the mean number of trichopteran larvae per sample did not change between adjacent sampling dates (all comparisons: 6df, k=3, P>0.10).

When the whole season was considered, the abundance of *Hyalella* in treatment ponds was reduced relative to that in control ponds. Only in the shallow zone, however, did this decline approach significance (F=2.39, 4,24df, P=0.08). Comparisons of means indicated no change in abundance in shallow zones of control ponds (all comparisons: 24df, k=5, P>0.10), but in the treatment ponds, a significant reduction occurred between pre- and post-spraying (t'=3.61, 24df, k=5, P<0.01); this reduction was approximately seven-fold, from 22.9 to 3.1 individuals (mean, backtransformed from LOG(x+1)) (Fig.II-5A). This reduction was sustained through May 1987 in the shallow zones of treated ponds (all comparisons: 24df, k=5, P>0.10) which suggests that populations of *Hyalella* had not recovered 10 months after exposure to carbofuran. In the deep zones of the treatment ponds a trend towards reduced numbers of this amphipod, following spraying, (Fig.II-5B) was similar to that in the shallow zone, although low mean abundances and high residual variances prevented detection of a statistically significant reduction. In the deep zones of the control ponds, *Hyalella* abundance suggested a slightly increasing trend throughout the study, whereas in treated ponds the overall trend was the opposite, mainly because of the August mean value which fell relative to that in the control ponds (Fig.II-5B). This overall decline in the treatment ponds reflects the drop in abundance recorded in ponds T3 and T4 where mean numbers (±1SE, n=4) in the deep zone fell from 9.8±2.1 and 59.3±11.9 individuals per sample pre-spraying to 2.0±4.1 and 0.8±0.5 post-spraying. Initial concentrations of carbofuran in these two ponds were higher than in pond T1 in which the abundance of this amphipod in the deep zones was relatively constant from July (5.0±2.1 individuals per sample, mean±1SE, n=4) through August (7.3±3.0).

Over the whole season, the abundance of coenagrionid nymphs

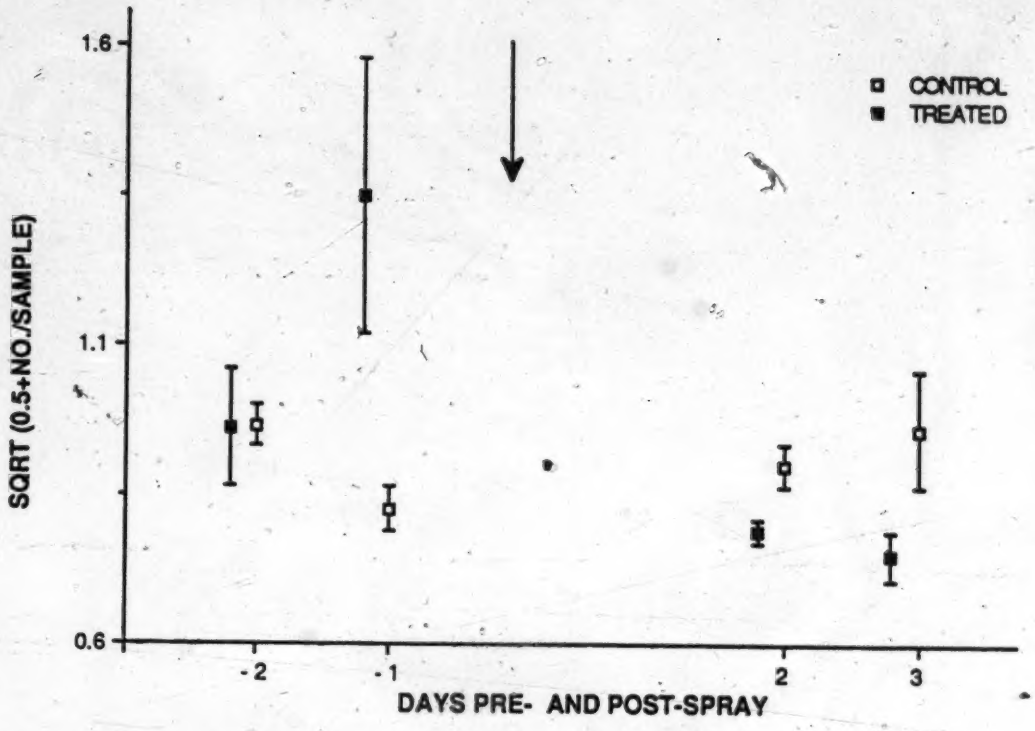

Fig. II-4. Abundance (mean±SE) of trichopteran larvae in two control ponds (C2 and 3) and two treated ponds (T2 and 4). Samples taken 1 and 2 days before spraying and 2 and 3 days after spraying. Carbofuran spraying date (July 23) indicated by arrow. Symbols offset for clarity.

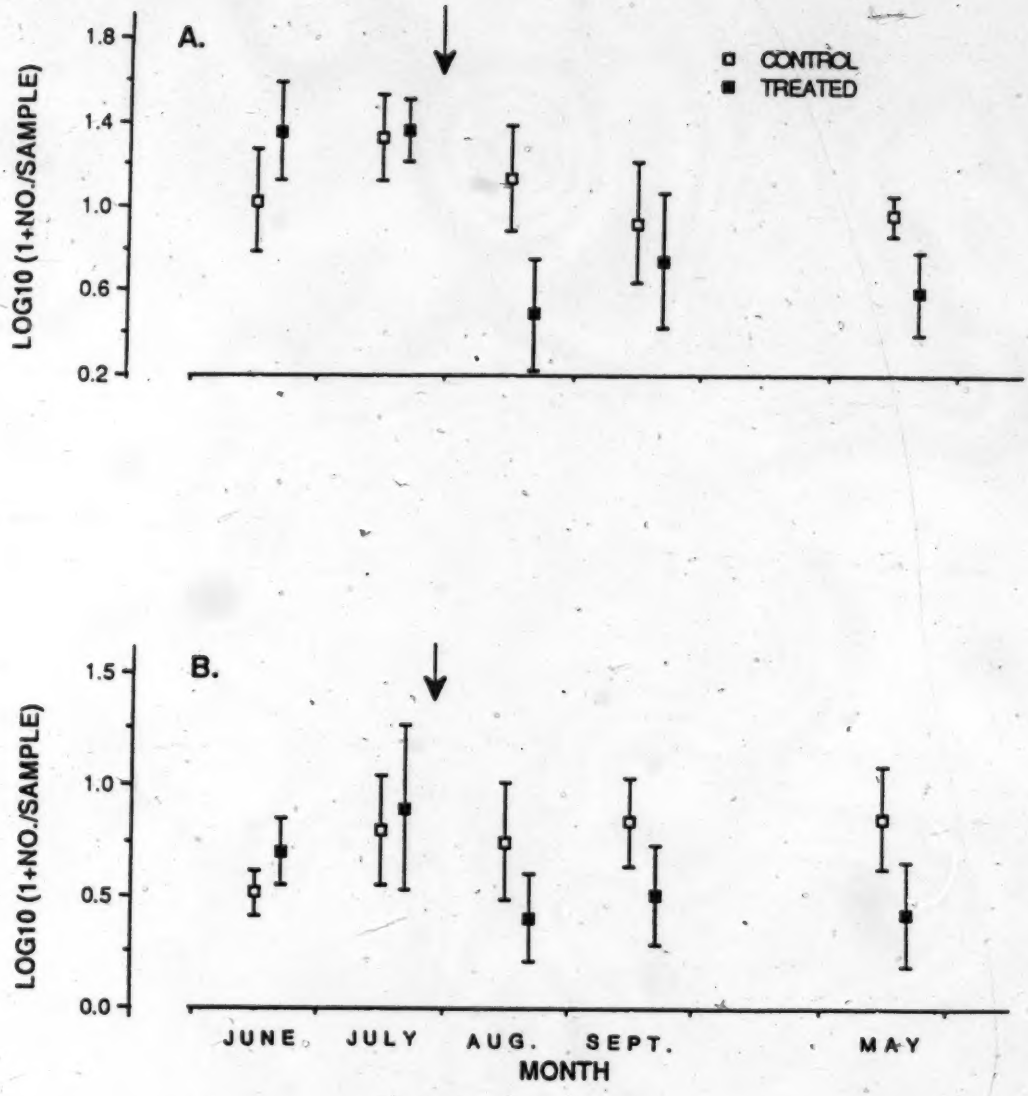

Fig. II-5. Abundance (mean±SE) of *Hyallela* in shallow (A) and deep (B) zones of four control ponds and four treated ponds from June, 1986 until May, 1987. Week of carbofuran spraying (July 23-30) indicated by arrow. Symbols offset for clarity.

was relatively similar in treated and control ponds (Fig.II-6) with one exception: in the treated ponds, the mean abundance of coenagrionid nymphs increased significantly between August, 1986 (3.2 individuals per sample) and May, 1987 (8.7 individuals per sample) (t'=3.55, 24df, k=5, P<0.01). This contrasts with the control ponds where there were no significant differences in mean abundance among any of the post-treatment months (3.4 individuals per sample in August, 5.2 in September and 4.4 the following May) (all comparisons: 24df, k=5, P>0.10).

In neither larval Chironominae (Fig.II-7) nor the ephemeropteran nymph *Caenis* (Fig.II-8) were differing seasonal patterns of abundance recorded in the treated and control ponds. The absence of a significant T X D interaction effect for both taxa (Chironominae: F=0.88, 4,24df, P=0.49; *Caenis* shallow zone: F=0.59, 4,24df, P=0.67; *Caenis* deep: F=0.06, 4,24df, P=0.99) indicated that neither of these taxa was overtly affected by exposure to carbofuran. Comparisons among monthly mean abundances within a group of ponds indicated that Chironominae larvae increased in control ponds most noticeably between August and September (t'=2.67, 24df, k=5, P=0.07), whereas in treated ponds, this group increased continuously between August 1986 and May 1987 (t'=3.55, 24df, k=5, P<0.01). Abundance of *Caenis* declined slightly to seasonal lows in July in both treated and control ponds and increased thereafter (Fig.II-8).The mean abundance of *Caenis* increased significantly in the shallow zones of control ponds between August1986 and May 1987 (t'=2.96, 24df, k=5, P=0.04). In the treated ponds the mean abundance of *Caenis* increased only slightly during the post-treatment phase of this study. This increase was not statistically significant (all comparisons: 24df, k=5, P>0.10). Nevertheless, throughout the post-treatment phase of the study, the mean abundance of *Caenis* in treated ponds was very similar to that in control ponds (Fig.II-8).

Despite the decrease in trichopteran larvae demonstrated in the samples taken on the second and third days after spraying (Fig.II-4), their seasonal abundance in the treated ponds was not significantly lowered below that in the control ponds as indicated by the absence of a significant T X D interaction effect (F=1.46, 4,24df, P=0.24). Trichopteran larval abundance reached seasonal lows in both treated and control ponds in late July just before application of the insecticide (Fig.II-4 &9). Thereafter they increased and their

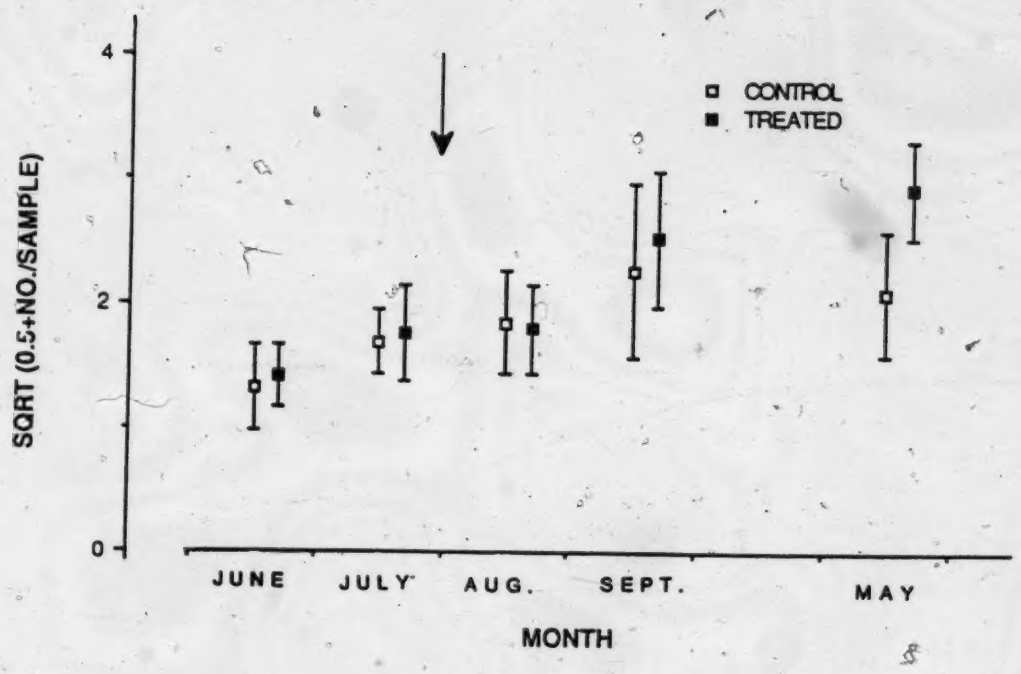

Fig. II-6. Abundance (mean±SE) of coenagrionid nymphs in four control ponds and four treated ponds from June, 1986 until May, 1987. Week of carbofuran spraying (July 23-30) indicated by arrow. Symbols offset for clarity.

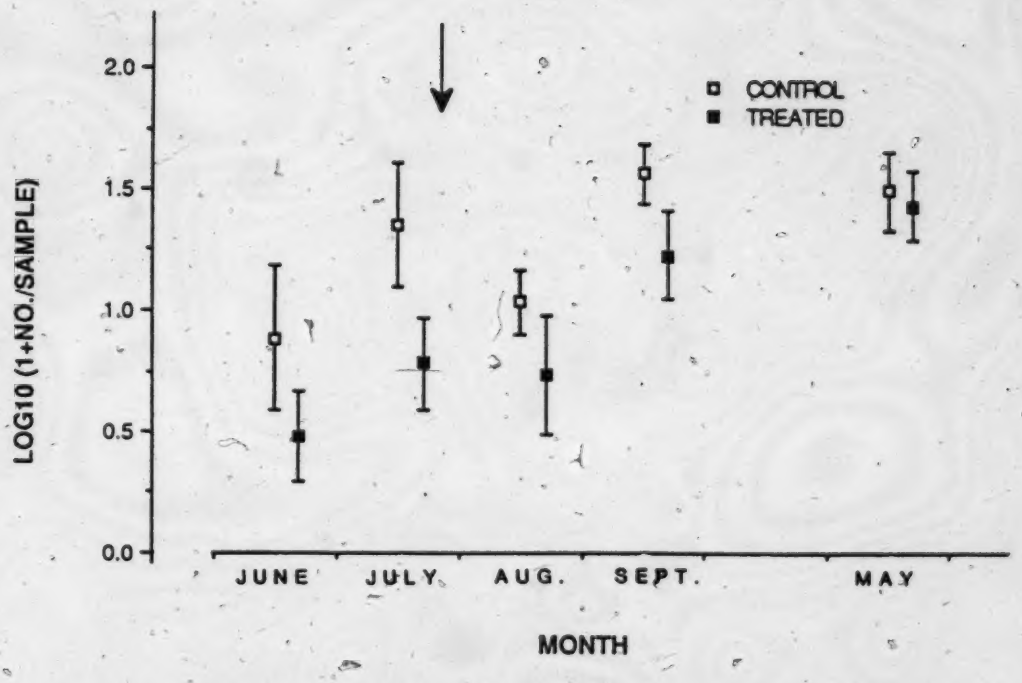

Fig. II-7. Abundance (mean±SE) of Chironominae larvae in four control ponds and four treated ponds from June, 1986 until May, 1987. Week of carbofuran spraying (July 23-30) indicated by arrow. Symbols offset for clarity.

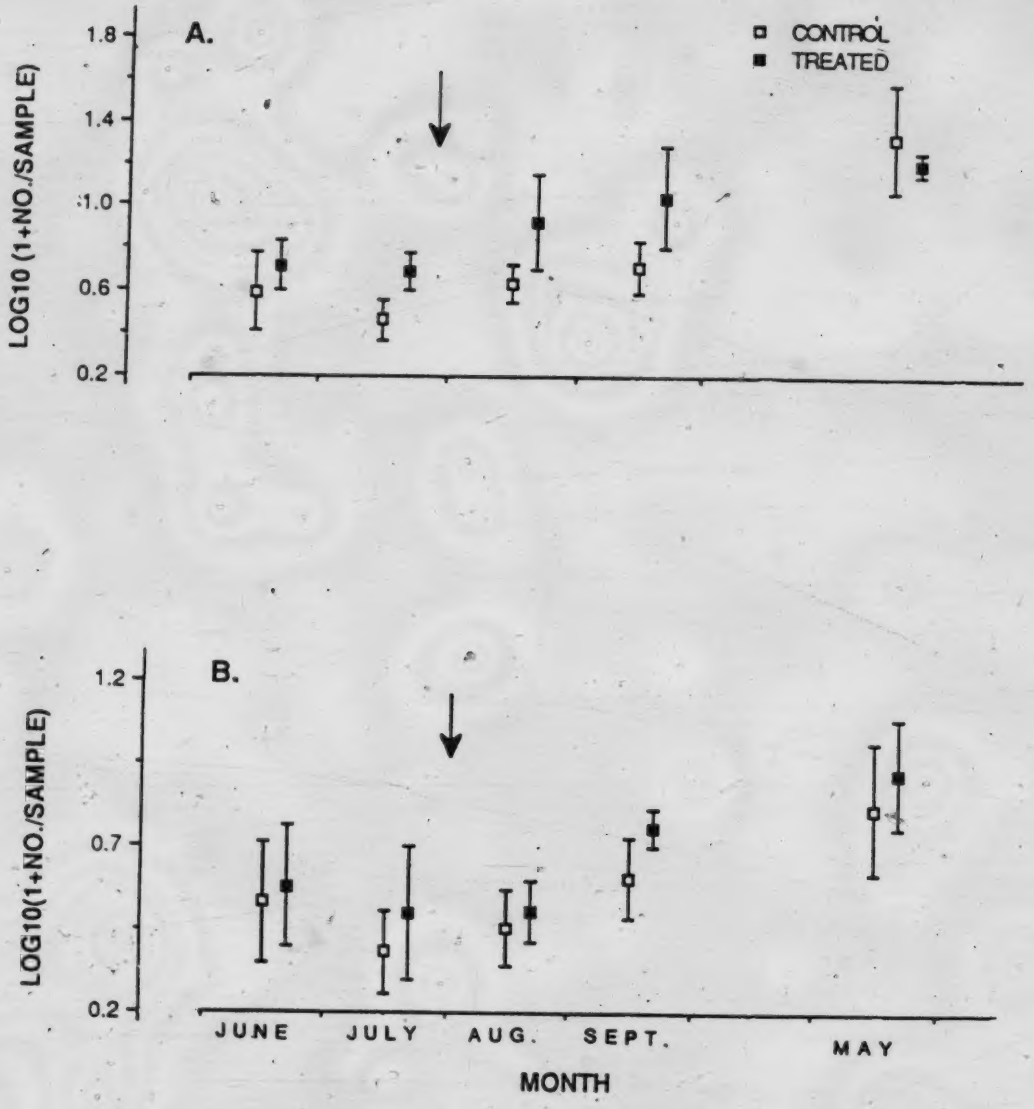

Fig. II-8. Abundance (mean±SE) of *Caenis* in shallow (A) and deep (B) zones of four control ponds and four treated ponds from June, 1986 until May, 1987. Week of carbofuran spraying (July 23-30) indicated by arrow. Symbols offset for clarity.

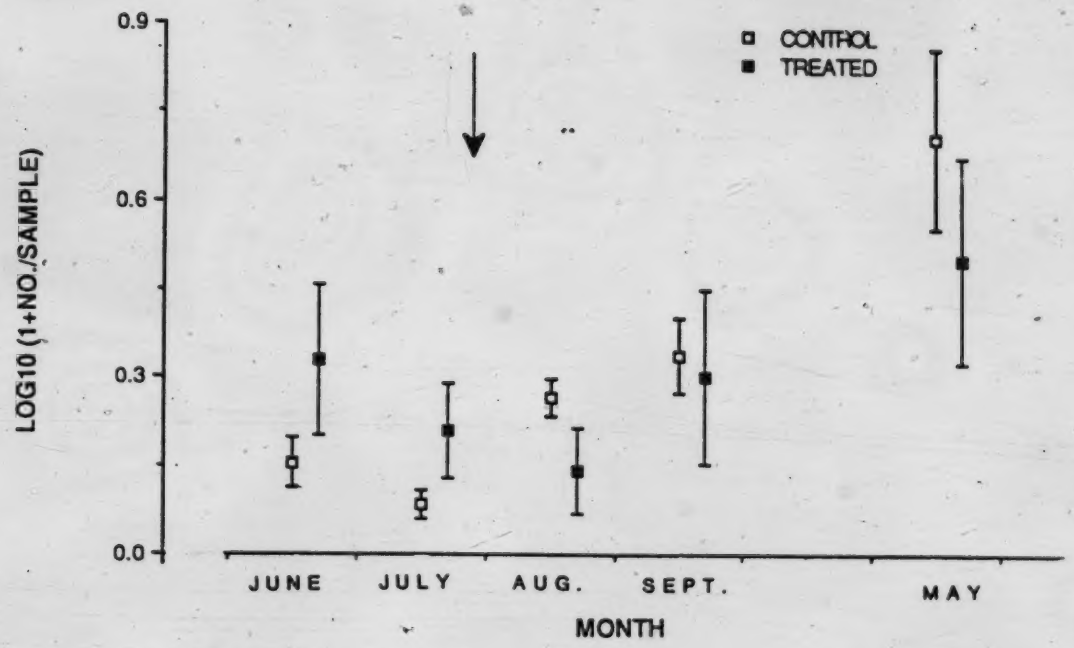

Fig. II-9. Abundance (mean±SE) of trichopteran larvae in four control ponds and four treated ponds from June, 1986 until May, 1987. Week of carbofuran spraying (July 23-30) indicated by arrow. Symbols offset for clarity.

numbers peaked in May, 1987 in both treated and control ponds. Over the whole season, nine genera representing five families were encountered in the study ponds. There were three leptocerids (*Mystacides, Triaenodes*, and *Oecetis*), three limnephilids (*Limnephilus, Nemotaulius* and *Anabolia*) and one phryganeid (*Agrypnia*) in addition to *Molanna* (Molannidae) and *Polycentropus* (Polycentropodidae). Only larvae of *Triaenodes, Limnephilus, Molanna* and *Agrypnia* were present at the time the ponds were sprayed. The low abundance of larvae coupled with the absence of several genera from the ponds at the time they were sprayed made it unlikely that carbofuran could exert a strong seasonal effect on larval abundance of Trichoptera. Nevertheless, it is noteworthy that mean abundance of trichopteran larvae was higher in the treated ponds than in the control ponds in June and July before spraying, whereas it was consistently lower during each sampling period after spraying (Fig.II-9). This trend in the data can be attributed, in part, to the relative post-treatment scarcity in abundance in treated ponds of those genera which were present in samples taken from the study ponds at the time of spraying. In the treated ponds, 94 larvae of the four genera represented in samples taken on the days immediately before and after spraying were obtained during the pre-treatment phase of the study examining the seasonal effects. In the control ponds 38 such larvae were collected during the same period. During the entire post-treatment phase of the study, only 47 such larvae were collected in treatment ponds while 216 were collected in samples from control ponds. A two by two contingency table analysis taking into account the total pre-spray and post-spray abundances of these four genera in both treated and control ponds indicated that their post-spray abundances were independent of their pre-spray abundances (Chi-square=109.0, 1df, $P<0.005$). On the other hand, when only those genera which were absent in samples taken around the time of spraying were considered, pre-spray and post-spray abundances were not independent of one another in treated and control ponds (Chi-square=3.6, 1df, $P>0.50$). There were large increases in this group of trichopteran larvae during the post-treatment phase of the study in both treated and control ponds (from 23 to 169 and from 7 to 140 larvae in treated and control ponds respectively). This is similar to the increase in the control ponds exhibited by the genera present in the ponds at the

time they were sprayed but is the opposite of the pattern shown in the treated ponds by this group. These data suggest that the reduction in trichopteran larval abundance in treated ponds relative to that in control ponds during the post-treatment phase of the study was due mainly to the deleterious effects of carbofuran on those genera present in the pond at the time of spraying. Unfortunately, small numbers of larvae per sample and high among pond variance prevent meaningful statistical analyses of each group of larvae separately according to the original experimental design which used repeated measures ANOVA.

DISCUSSION.

Fate and persistence: At 25°C, the estimated half-life ($t_{1/2}$) of carbofuran through hydrolysis in aqueous solution is 5 days at pH 8 and 0.8 days at pH 9 (NRCC 1979). Chapman and Cole (1982), on the other hand, calculated $t_{1/2}$ from the hydrolysis rate constant and found it to be 7 days at pH 8 and approximately 25°. Estimates of $t_{1/2}$ in the study ponds in which the pH was slightly less than 9 and mean temperatures were from 16-19°C (Table II-2) were slightly greater (30-74 hr) than those reported above. Perhaps, this was because average daily temperatures of the study ponds were lower than those used in the above studies. Also, since pH values were recorded during peak hours for photosynthesis, they do not account for potential nighttime reductions associated with the increase in concentration of CO_2, and may be biased upwards..

The rate of degradation was faster in pond T3 when compared to the other ponds. This suggests that carbofuran degradation rates in prairie ponds may be highly variable notwithstanding their apparently similar physico-chemical features.

The rate of loss of carbofuran from aquatic plants in the study ponds appeared to parallel the corresponding rates of loss of carbofuran from water. The relationship between degradation of pesticides and their resultant desorption from the sediments or other surfaces into the water column has been noted elsewhere (Hughes et al. 1980, Huckins et al.1986), and they suggested that this process maintained an equilibrium. In this study, it is possible that hydrolysis of carbofuran in the water column permitted its

desorption from plants into the water column to maintain an equilibrium.

The relatively low recovery of carbofuran from pond sediments agrees with earlier observations that it is unlikely to accumulate on pond bottoms (NRCC 1979) but, with the exception of pond T1, differs from the results of Klaassen and Kadoum (1979) who recorded carbofuran residues 2-8 times higher in the sediments than in overlying water of both their study ponds up to 3 days after application.

Carbofuran concentrations were, on average, 3-50 times higher in plants than in the surrounding water of the study ponds. Studies dealing with other pesticides have shown similar trends (Mulla et al. 1966a, Hurlbert et al. 1970, Rawn et al. 1982, Huckins et al. 1986). There was great variability in adsorption by plants among sites, and to a lesser extent, between ponds and dates. Carbofuran concentrations in plants ranged from 1.7 to 130 times greater than in the surrounding water. This variability may have implications for its bioavailability to invertebrates. It has been shown under laboratory conditions that, for hydrophilic pesticides such as carbofuran, bioconcentration of pesticides by benthic invertebrates appears to be unrelated to microhabitat (Muir et al. 1983). In spite of this finding, the possibility exists that under field conditions promoting rapid degradation of a hydrophilic pesticide such as carbofuran, epiphytic invertebrates which constitute the majority of macroinvertebrates in shallow, macrophyte-filled wetlands (Krull 1970, Voights 1976) may be subjected to relatively high levels of exposure as a result of adsorption-desorption phenomena at the plant-water interface. This is consistent with the suggestion in the literature that desorption of pesticides from sediments may result in temporarily higher concentrations in pore water or water closely associated with the sediments than in the overlying water resulting in greater bioconcentration of pesticides in invertebrates associated with sediments than in those in overlying water (Lynch & Johnson 1982, Muir et al. 1985). Thus, high within-pond variability in partitioning into plants could result in variability in exposure of epiphytic invertebrates. The phenomenon of microhabitat-induced effects on pesticide bioavailability needs to be more fully examined, especially as it relates to aquatic plants and their epiphytic fauna, such as the damselflies recorded in this study.

Effects on macroinvertebrates: The addition of carbofuran to the study ponds immediately reduced the abundance of *Hyalella* in the shallow zones and possibly, in the deep zones of treated ponds. This was not surprising given the sensitivity of *Hyalella* and other amphipods to several insecticides. Sharp reductions in *Hyalella* abundance have been noticed in field studies involving the carbamate insecticide carbaryl (Gibbs et al. 1984) and the organophosphate, diazinon (Arthur et al. 1983). Moreover, laboratory experiments evaluating the susceptibilities of aquatic organisms to pyrethroid insecticides have demonstrated that other freshwater amphipods are as sensitive or more sensitive than the most sensitive aquatic insects used in the experiments (Muirhead-Thomson 1978, Anderson 1982, Stephenson 1982).

The initial reductions in *Hyalella* abundance in the treated ponds persisted for the remainder of the summer and into the early part of the following spring indicating the absence of any density-dependent mechanism that would enable the populations to recover after the ponds were sprayed. This result is consistent with Cooper's (1965) observation that the intrinsic rate of population growth in *Hyalella* was not density-dependent within the constraints of density used in his study but was not consistent with the results of Wilder (1940) who showed that fecundity increased at low population densities while mortality was independent of density. Breeding pairs of *Hyalella* (in amplexus) were not observed in the study ponds after mid-July suggesting that reproductive activity was either very low or non-existent by the beginning of the post-spray period. While this observation is inconsistent with the evidence suggesting that *Hyalella* is capable of producing offspring into late summer in north temperate lakes (Cooper 1965, Mathias 1971, de March 1977), it may explain, nonetheless, the lack of any recovery in *Hyalella* abundance up to 10 months after spraying (Fig.II-5). While mortality rates are apparently not inversely-related to density (Wilder 1940), they may vary temporally according to the age structure of the population (Cooper 1965). Thus, a sudden pesticide contamination of a pond could result in the premature removal of an age class which is destined to die at a later date anyhow. Through age-related natural mortality, populations in unaffected ponds might eventually decline relative to those in contaminated ponds which suffer large-scale, sudden and non-age-

related mortality. There is some evidence of such a pattern in the shallow zones of treated and control ponds during the post-spray period (Fig.II-5A). However, in the deep zones, no such pattern emerged (Fig.II-5B). The abundance of trichopteran larvae declined in the treated ponds immediately after spraying. This result corresponded well with the observation of many stressed and dying phryganeid and limnephilid larvae on the surface of ponds T1 and T4 16 hr after spraying. Field studies have demonstrated the relative susceptibility of trichopteran larvae to organophosphate insecticides. Caddisfly larvae were more susceptible to chlorpyrifos than mayflies or midges (Macek et al. 1972). Arthur et al. (1983) placed trichopterans in a group along with four other taxa, which they considered to be most susceptible to diazinon.

Unlike *Hyalella*, the number of trichopteran larvae increased again following the initial depression in their abundance. By August, trichopteran abundance in treated ponds was only slightly less than pre-spray levels and had surpassed pre-spray levels by September. Overall, seasonal changes in trichopteran abundance in treated ponds paralleled those observed in the controls. Patterns of change in the study ponds were consistent with life-history information on caddisflies adapted to lentic, permanent water-bodies which, in general, indicates that larval populations are high during spring and early summer, decline as a result of pupation and emergence from late spring until mid-summer and increase again during the fall (Winterbourn 1971a, Winterbourn 1971b, Berté & Pritchard 1986). Thus a mid-summer application of a short-lived insecticide such as carbofuran, may not affect that portion of the trichopteran fauna of prairie ponds whose life-cycles are characterized by spring or early summer emergence and late summer egg-hatching. Species adapted to temporary water bodies, however, may be more prone to long-term effects since they do not emerge until later in the summer (Berté & Pritchard 1986). This may partially explain the failure of trichopteran larvae to repopulate the treated ponds at a rate similar to that recorded in the control ponds during the post-treatment phase of this study (Fig.II-9). Larval populations can be quite high early in the season; thus, the effects of an earlier date of insecticide application may be quite different from those observed in this study (Fig.II-9) since recolonization would depend heavily on adults dispersing from neighbouring ponds which were not affected.

The abundance of larval Chironominae was apparently

unaffected by exposure to carbofuran within the range of concentrations used (Fig.II-2). The toxicity of carbofuran to larval Chironominae varies widely with species and/or test conditions. The reported 48-hr EC50 for *Chironomus riparius* is 56 µg/L at a pH of 8.5-9.0 (Johnson 1986). *C. tentans* is apparently more sensitive with an LC50 estimated at 1.6 µg/L under unspecified test conditions (Karnak & Collins 1974). Acute mortality in the field was 88% for *Goeldichironomus holoprasinus* and 90% for *Chironomus stigmatarus* at an application rate of approximately 94 µg/L (calculated from Mulla & Khasawinah 1969). In my study, neither the species nor generic composition of the Chironominae were quantified. However, several larvae collected from ponds T2 and T4 during the days just before and after spraying were identified to genus. Larvae from pond T2 were predominately *Tanytarsus*, while those from pond T4 were predominately *Paratanytarsus, Glyptotendipes*, and *Chironomus*. It is possible that these groups were not as sensitive to carbofuran as *C. tentans* which suffered heavy mortality when held in small cages in the treated ponds at the time of carbofuran spraying (Chapter IV).

Over the whole season, the abundance of Chironominae larvae was not affected substantially by the mid-summer application of carbofuran. Nevertheless, a slightly greater increase in their abundance was recorded in the treated ponds than in the control ponds during the post-treatment phase of the study (Fig.II-7). Kennedy et al. (1970) observed a similar pattern in ponds treated with methoxychlor, wherein numbers of chironomid larvae had increased to a greater degree than in control ponds 28 days after spraying. They suggested that a reduction in competitors or predators and/or the organic enrichment resulting from the large-scale die-off of invertebrates immediately following treatment may have accounted for the disproportionate increase. In this study, it is possible that the slight, disproportionate increase in the treated ponds relative to the control ponds resulted from the reduction in a potential competitor such as *Hyalella*, which, like many of the Chironominae, is a benthic herbivore and detritivore (Hargrave 1970). Nevertheless, since this difference was slight and non-significant, the possibility that it might have been due to sampling variability alone cannot be discounted.

There was no detectable reduction in coenagrionid nymphs

following carbofuran spraying. I am unaware of any information on insecticide toxicity to coenagrionid nymphs. However, late-instar nymphs of another damselfly, *Lestes congener* (Lestidae), appear to be more resistant to the organophosphates, diazinon and malathion, than other groups of insects with 24 and 96 LC50 values of 300 and 50 µg/L (Federle & Collins 1976 in Sheehan et al.1987). Also, *in-situ* cage experiments discussed in Ch. IV demonstrate that survival was higher for this group than for either *Gammarus lacustris* or *C. tentans* held in separate cages, although some carbofuran-induced mortality probably did occur. While the cage experiments suggest the possibility that some mortality may have been due to carbofuran, it appears that concentrations used in this experiment were not high enough to yield detectable reductions in coenagrionid populations in the treated ponds.

During the post-treatment phase of the study, the abundance of coenagrionid nymphs in control ponds remained fairly stable whereas, in treated ponds, they increased (Fig.II-6). Whether this difference was a secondary effect of carbofuran remains unclear. On the one hand, it may be attributed to improved survival and/or increased recruitment in the treated ponds during the post-treatment phase of the study. While prey availability or the lack thereof is not likely to affect mortality associated with starvation, it has been found to influence developmental rates of odonates (Lawton et al. 1980). These authors suggest that overall fitness should, therefore, be enhanced under conditions of optimal prey availability. Thus, increases in abundance of potential prey such as cladocerans, copepods or chironomids as a result of certain competitors or predators being selectively killed by carbofuran (*sensu* Hurlbert 1975) could conceivably improve some aspect of fitness (either fecundity or the probability of survival) in coenagrionid nymphs and thus lead to increases in coenagrionid abundance in the treated ponds. Other possible explanations are that the availability of adults dispersing from other ponds or some variable aspect of life history tactics of coenagrionid species in this study resulted in greater recruitment in some of the treated ponds than in the controls. In fact, only one treatment pond showed a large increase between August 1986 and May 1987. In pond T2, abundance of damselfly nymphs rose from 3.8±1.1 in August to 14.3±4.1 the following May (mean±1SE, n=8, based on original data).

Increases in the three other treatment ponds were more modest: from 0.4±0.1 to 5.1±2.3 in pond T1; from 2.0±0.6 to 4.3±1.1 in pond T3; and from 6.3±2.0 to 10.5±3.2 in pond T4. During the same period of time, damselfly abundance increased marginally in two control ponds (from 8.4±1.8 to 12.0±2.4 and from 0.5±0.0 to 2.0±0.4) and decreased marginally in the other two. Thus, it is apparent that the significant increase in mean abundance in the treated ponds is due mainly to the increase in pond T2 which happened to have received the second lowest concentration of carbofuran in this study. It is possible that some other factor independent of carbofuran, such as increased dispersal of adults to or increased egg deposition and hatching during the post-spray period in pond T2 and perhaps to a lesser extent in ponds T1 and T4, might have accounted for the differences between treatment and control ponds in rates of increase of damselflies. It is noteworthy that a great deal of asynchrony in emergence phenologies and timing of egg deposition has been demonstrated for two coenagrionid species commonly occurring in central Alberta (Baker & Clifford 1981, 1982). If such asynchrony existed among damselfly populations in the study ponds, temporally-different patterns of population change could result.

Caenis abundance declined through emergence and reached seasonal lows by late July. Their abundance began to increase again in August after the effects of carbofuran had dissipated. Thus, there was little opportunity for carbofuran to have a negative effect on *Caenis*. On the contrary, since both *Caenis* and *Hyalella* are considered to be deposit feeders on epibenthic or epiphytic algae (Hargrave 1970, Cummins 1973), it seemed possible that the carbofuran-induced reduction in *Hyalella* might have reduced competition and/or increased food availability enough to permit *Caenis* abundance to increase at a faster rate in treated ponds. While previous studies have indicated that competition can play a role in structuring benthic communities (Hall et al. 1970, Cantrell & McLachlan 1977) and that pesticide-induced changes in planktonic (Hurlbert et al. 1970, Crossland & Elgar 1983, Kaushik et al. 1985) and benthic (Sanders et al. 1981) communities can result in large increases in abundance of resistant groups, I did not detect such a secondary effect on *Caenis* in this study. Notwithstanding the seasonal impact on *Hyalella* which had been one of the numerically dominant taxa in the ponds before spraying, it is possible that the benthic communities in the treated ponds were not sufficiently

changed by carbofuran to allow detection of any secondary effects on *Caenis*. However, since 50% of the total number of invertebrates recorded in this study were not considered in the analysis, secondary effects on some taxa may not have been detected.

In summary, in the short term carbofuran reduced *Hyalella* and trichopteran larval abundance in the treated ponds. *Hyalella* abundance remained low into the following spring while emergence, dispersal, breeding and egg hatching phenologies of the caddisflies allowed their partial recovery within a matter of weeks. There was no evidence of a direct reduction in Chironominae larvae or coenagrionid nymphs following spraying and neither these groups nor *Caenis* appeared to be indirectly affected over the whole season; the coenagrionid data were equivocal on this point and may, in fact, have had increases in their post-spray fecundity or survival. Application of carbofuran at a time of year when larval populations of susceptible groups (e.g. caddisflies) were much higher could have led to quite different results.

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CHAPTER III

THE EFFECTS OF CARBOFURAN ON MACROINVERTEBRATES IN A PRAIRIE POND: AN ENCLOSURE EXPERIMENT

INTRODUCTION.

In discussing the difficulties of designing field experiments, the objectives of which were to assess the impact of chemicals on aquatic systems, Stephenson and Kane (1984) emphasized the complexity of aquatic systems and the resultant difficulty of finding or creating suitable replicates. Adequate replication depends, in part, on finding experimental units that are sufficiently similar so that inherent variability does not mask experimental effect and in selecting units to which a given treatment level can be applied equally without appreciable error.

Interpretation of the multi-pond experiment discussed in Chapter II was often made difficult because of both these factors. There was high between-pond variability in abundance of various taxa (Chapter II), a characteristic of invertebrate assemblages noted elsewhere for prairie ponds (Bartonek & Hickey 1969). Another complicating factor in the multi-pond study was that the resulting concentrations of carbofuran in the treatment ponds were not identical and, in fact, ranged from about 9 to 32 µg/L 16 h after spraying. One way to overcome these problems is to use small enclosures within a pond or lake (Hodson & Millard 1978, Solomon et al. 1980, Stephenson & Kane 1984). Several experiments which used small enclosures in ponds and lakes, have been conducted recently to evaluate the effects of insecticides on zooplankton assemblages (Stephenson & Kane 1984, Kaushik et al. 1985, Stephenson et al. 1986), on fish (Shires 1983) and on benthic invertebrates (Shires 1983, Stephenson & Kane 1984) . This study used enclosures to examine the effects of carbofuran on benthic macroinvertebrates in a prairie pond. I predicted that the enclosure technique would improve the repeatability of the experiment and thus increase the level of confidence in the results. In addition to improved repeatability, I evaluated the effects of carbofuran on a wider range of common pond taxa than in the multi-pond study. Also, since invertebrate biomass may be as important (Kaminski & Prince 1981,

Murkin & Kadlec 1986) or perhaps more important than abundance (Pehrsson 1984) in determining pond selection by breeding and brood-rearing ducks as well as growth rates of ducklings (Hunter et al. 1984), and since insecticides may be more toxic to smaller individuals within a species (Sanders 1969), a final objective was to evaluate the impact of carbofuran on both abundance and biomass of macroinvertebrates.

STUDY AREA.

The pond used in this experiment was located near Sherwood Park, Alberta (53°33'N 113°15'W). It was semi-permanent with greater than 5% emergent vegetative cover and was designated as Type IV-B-3 according to the classification system of Stewart and Kantrud (1971). The pond was dominated by open water in which an abundance of submersed and floating vegetation grew, primarily *Lemna trisulca, L. minor* and *Ceratophyllum demersum*. It was surrounded by a thick band of cattail (*Typha sp.*). Some physico-chemical parameters of the pond water are outlined in Table III-1.

There was one inlet to the pond which had stopped flowing by early summer. A culvert provided an overflow out of the pond into a ditch. It could be manually opened or closed allowing partial control of water levels.

METHODS.

Twenty-one cylindrical, water-tight enclosures 1.8 m in diameter X 0.6 m high were used in this experiment. They consisted of top and bottom 1.8 m diameter rings made from flexible 3.75-cm polyvinyl chloride (PVC) pipe. Each section of pipe was formed into a circle, the ends being joined by a coupling. Several 2-cm thick X 10-cm long pieces of styrofoam were glued to each upper ring enabling it to float on the water surface. Through each ring four holes,1.5 cm in diameter, were drilled at equidistant intervals. Lengths of reinforcing bar (rebar), 1.5 m long, were passed through each set of corresponding holes in the top and bottom rings of each enclosure. The bottom ring of each enclosure was held in place between two 5-cm long cotter pins, each of which passed through

Table III-1. Some physico-chemical features[1] of water (Mean±1SE.) within enclosures based on samples taken after the treatment date (July 15).

Variable	Control	5 µg/ L	25 µg/ L
Dissolved O_2 (mg L^{-1})	11.7±1.1[2]	7.3±1.4[3]	9.1±2.2[3]
pH	9.2[2]	9.1[3]	8.9[3]
Turbidity (NTU)[4]	3.7±0.8[2]	4.5±0.8[3]	4.8±0.9[3]
Conductivity (µmhos cm-1)	428±7[2]	432±13[3]	431±13[3]
TKN[5]	2.88±0.24	3.23±0.05	3.99±0.88
Alkalinity[6]	200±4	200±7	202±10
Sodium[6]	30.0±0.1	28.9±0.0	30.0±0.9
Potassium[6]	26.5±4.1	26.2±1.8	27.5±2.8
Calcium[6]	48.3±2.7	45.7±2.5	49.2±1.3
Magnesium[6]	19.1±0.5	19.2±0.2	20.3±0.9
Sulphate[6]	50.7±6.3	53.6±2.3	50.2±1.9

1. Dissolved oxygen, pH, turbidity, and conductivity determined as in Chapter 1. Other variables determined according to Alberta Environmental Centre (1977) by Chemex Labs Alberta (1984) Ltd.
2. n=7 enclosures sampled in each group. Average of 2 dates (Aug.15 & Sept.8).
3. n=3 enclosures sampled in each group. Average of 2 dates (Aug.15 & Sept.8).
4. Nephlometric tubidity units.
5. Total Kjeldhal nitrogen (mg/L). n=3 enclosures sampled in each group. Samples collected Aug.15.
6. mg/L. n=3 enclosures sampled in each group. Samples collected Aug.15.

holes drilled through the rebar. The holes were drilled about 50 and 60 cm above the bottom end of each length of rebar, enabling the bars to be pushed into the substrate to a depth of 50 cm. A double layer of 6 mm thick clear polyethylene was glued and clamped to the ring and rebar frame. The seam in this polyethylene covering was sealed with a 1.5-m long X 5-cm wide strip of rubber, attached to the polyethylene with silicone sealant and staples.

Once the enclosures were built, they were placed in the pond at randomly selected sites where the water was from 40-50 cm deep. In order to seal each enclosure to the pond bottom, the lower 50-cm long sections of rebar were driven into the substrate until the bottom ring came in contact with the bottom sediments. The substrate was then manually pushed up against the outside of the bottom of each enclosure to make certain there were no gaps between the bottom ring and the pond bottom. Finally, each enclosure was encircled by a poultry wire exclosure, 2.2 m in diameter X 1 m high, to prevent muskrats (*Ondatra zibethica)* from damaging the enclosures. All enclosures were in place by July 7, 1987.

The 21 enclosures were assigned at random to one of three treatments for a total of seven enclosures per treatment. The three treatments were control (receiving no carbofuran), 5-µg/L carbofuran, and 25-µg/L carbofuran. These levels encompass a range of concentrations which could occur in a shallow prairie pothole (average depth ≤1 m) following accidental contamination while spraying adjacent fields for grasshopper control at the recommended rate of 140 g/ha.

Enclosure volumes were calculated from diameter and water depth data recorded on July 14. On July 15, appropriate quantities of carbofuran as Furadan 480 Flowable® were weighed on an electronic Mettler H10 balance. These quantities were subsequently diluted in 500 mL of slightly acidified water (pH 5-6). Enclosures targeted for treatment were treated within 2 h of weighing the pesticide. Treatment consisted of pouring the 500-ml solutions into the enclosures and agitating the water column with a paddle to ensure mixing. Water temperature and pH were recorded in each enclosure at the time of treatment.

Approximately 2 h after application, 1-L water samples were collected from three control, three 5-µg/L, and three 25-µg/L enclosures as well as at three sites located outside the 5-µg/L and

25-µg/L enclosures. All enclosures sampled were selected at random. The water samples were immediately acidified with H_2SO_4. All samples were analyzed for carbofuran residue within 24 h according to the methods described in Chapter II.

Invertebrates were sampled on six dates during the summer: two pre-application (July 8 and 14), and four post-application (July 20, 26, August 16, and September 8). A stovepipe sampler was used to collect invertebrates according to a technique modified from Rosenberg (1973) and described in detail in Chapter II. Over the whole season, less than 5% of the total water volume within any given enclosure was removed by this sampling technique. Moreover, less than 20% of the total sediment surface area within the enclosures was disturbed by this technique. Thus, I have assumed that the sampling technique did not deplete benthic invertebrates to a significant extent. All samples were preserved in 70% ethanol in the field. The preservative also contained a small amount of rose bengal stain which colored the animals but not the detritus, thus facilitating the separation of invertebrates from the sediments and plant constituents of the samples.

In the laboratory, samples were washed through a series of sieves of minimum mesh size 500 µm. A sugar flotation technique (Flannagan 1973) was used to aid in sorting the samples. Invertebrates were grouped according to taxonomic category, counted, rinsed in water to remove any adhering sugar, placed in pre-weighed aluminum weighing boats and dried for 24 h at 105°C to obtain a dry-weight estimate (Driver et al. 1974). No attempt was made to correct dry-weight estimates for preservative-induced weight loss. However, since weight loss stabilizes about 30 days after preservation (Howmiller 1972), and since most samples were not processed for at least one month after being collected, differential weight loss among samples was not likely to have been a problem when comparing biomass estimates for individual taxa at different treatment levels. All macrobenthic invertebrates were identified to species or genus where possible. Midge larvae (Chironomidae) were identified only to subfamily except for large larvae of the genus *Chironomus*. Because of difficulties in identifying small damselfly nymphs to genus, this group was identified only to family (Coenagrionidae). Zooplankton were not considered in this study.

To stabilize variances, data were transformed using either square-root (SQRT), fourth-root (4THRT) or logarithmic transformations (Downing 1979). The effects of carbofuran on individual taxa were analyzed using a repeated measures ANOVA (Winer 1971, p.519). The main effect was treatment level (control, 5-µg and 25-µg carbofuran), and the subplot effects were sampling date and treatment X sampling date interaction (T X D). When interaction, date and/or treatment effects were significant ($P \leq 0.05$), planned mean comparisons among all post-treatment sampling dates and between successive pre-treatment and the last pre-treatment and first post-treatment dates within each treatment level were done according to Winer (1971, p.384) using the Dunn-Sidak method (Sokal & Rohlf 1981, p.242) to adjust the experimentwise error rate. The purpose of such tests was to determine if the changes recorded over time in abundance or biomass for a given taxon were dissimilar among different treatment levels.

RESULTS

Based on water samples taken 2 h after application, carbofuran concentrations in the treated enclosures approximated the target levels of 5 µg/L and 25 µg/L. Moreover, there was no detectable leakage of carbofuran from the experimental enclosures and none of the sampled control enclosures was accidentally contaminated (Table III-2).

Forty-one taxa were identified in samples taken from the enclosures. However, most taxa were rare and some were absent from a given treatment level altogether (Appendix II). I subjectively determined that only eight taxa were sufficiently common in samples taken from the enclosures to warrant analysis: *Hyalella azteca*, Chironominae larvae, Tanypodinae larvae, the snails (Gastropoda) *Physa* and *Helisoma*, the glossiphoniid leech *Helobdella stagnalis*, the mayfly nymph *Caenis* (Caenidae) and finally, damselfly nymphs of the family Coenagrionidae. These taxa accounted for 97% of the total number of invertebrates recorded in samples taken from the enclosures throughout this study.

Of the four taxa which were analyzed in this study as well as in the 1986 multi-pond study discussed in Chapter II, three

Table III-2. Temperature, pH and concentrations of carbofuran in water column approximately 2 h after application.

Treatment Level	pH (Mean, n=7)	Temperature (°C) (Mean±1SE, n=7)	Concentration (µg/L) (Mean±1SE, n=3)
Control	9.0	21.0±0.4	ND[1]
5 µg/L	9.0	21.0±0.4	6.3±1.5
25 µg/L	9.1	21.5±0.2	22.5 (13-32)[2]
OTE[3]	8.9	21.0±0.4	ND[1]

1. Residue levels were not detected (ND). Detection limit=0.5µg/L.
2. One sample was accidentally contaminated and subsequently discarded. Values represent mean and range based on 2 samples.
3. Samples from outside treated enclosures. n=3.

exhibited lower variability attributable to enclosures than that attributable to ponds in the multi-pond study (Appendix III), confirming the value of this approach for studies requiring low variation among ponds (or enclosures). More importantly for this study, however, residual variability (i.e. the variance resulting from the interaction between date and enclosure (or pond), averaged over the three treatment levels) was reduced only for *H. azteca* (hereinafter referred to as *Hyalella*) by the enclosure approach, while it was higher for the other three taxa. Within the context of this experimental design, residual variation was of greater relevance than that attributable to ponds or enclosures, since it was this source of variation with which that attributable to the interaction between treatment and date was compared in evaluating the presence or absence of an acute or primary effect of carbofuran. Thus, in this study, the enclosure approach was not a major improvement when compared with the multi-pond approach.

In evaluating the effects of carbofuran on the various taxa in this study, it was first necessary to establish that changes between the two pre-treatment sampling dates (i.e. in the absence of carbofuran) were parallel to those in the controls. A carbofuran-induced decline would then be indicated by a decline in abundance or biomass between the last pre- and first post-treatment sampling dates in the 5-µg/L or 25-µg/L enclosures which was not matched in the controls. During the post-treatment period, changes in abundance or biomass of macroinvertebrates in the controls which were not matched by those at either concentration of carbofuran might indicate secondary effects (*sensu* Hurlbert 1975).

In the control ponds, *Hyalella* abundance and biomass increased gradually until Aug. 16 and declined slightly thereafter (Fig.III-1). The pattern of change in the 5-µg/L enclosures was similar to that seen in the controls, while in the 25-µg/L enclosures, *Hyalella* abundance and biomass declined following spraying, remained low until August 16 and recovered somewhat by Sept. 8 (Fig.III-1). Changes over time in both the abundance and biomass of *Hyalella* were not consistent among treatment levels as evidenced by the significant interaction term (Table III-3). In the control and 5-µg/L enclosures, no significant differences were noted between the two pre-treatment, between the last pre- and first post-treatment, and among all post-treatment sampling dates (Appendix IV), indicating that carbofuran, at 5 µg/L, did not have any

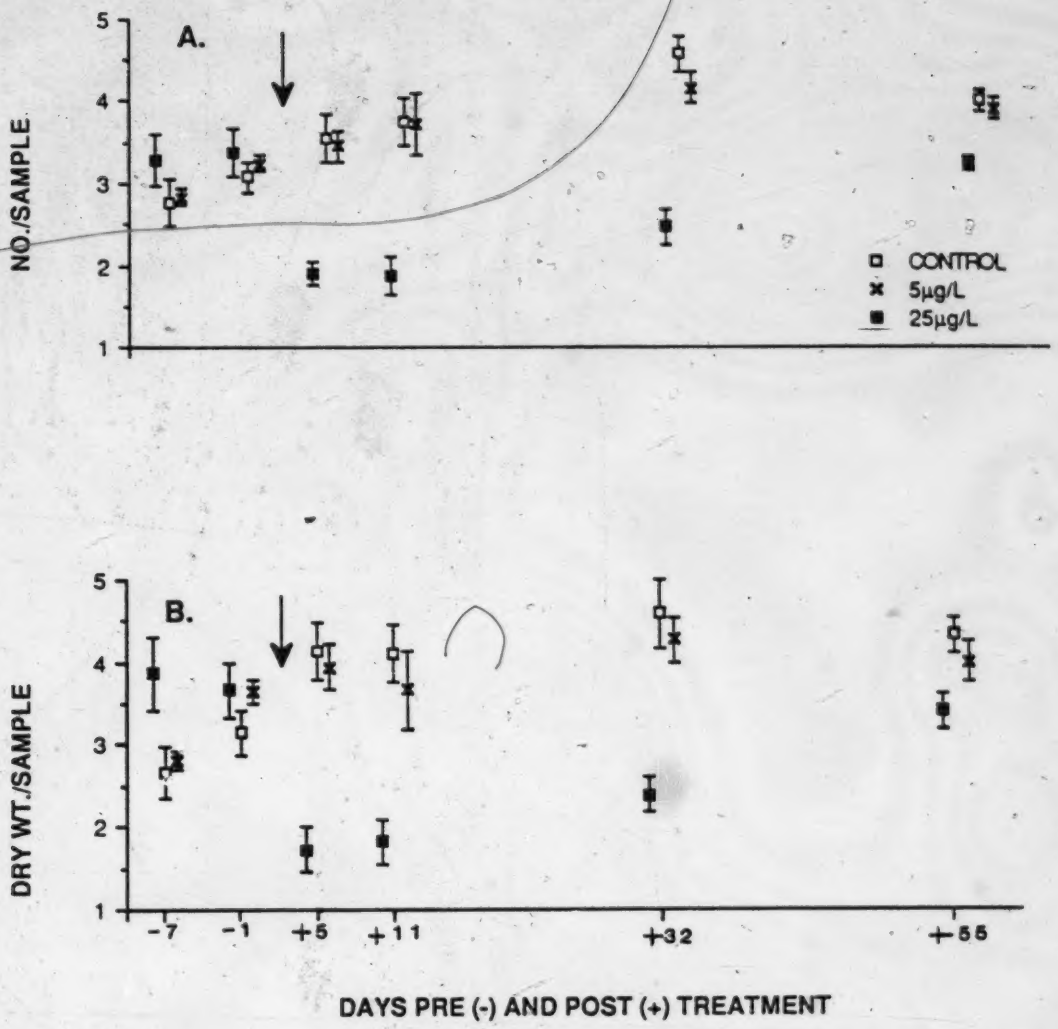

Fig. III-1. *Hyallela azteca*: mean (±SE) (A) abundance (fourth root transformed) and (B) biomass (mg) (fourth root transformed) in control, 5µg/L, and 25µg/L enclosures (n=7 for each group) on 6 sampling dates during summer, 1987. Treatment date (July 15) indicated by arrow. Absicca co-ordinates: -7, -1, +5, +11, +32, and +55 correspond to July 8, 14, 20, 26, Aug. 16, and Sept. 8 respectively. Symbols slightly offset for clarity.

Table III-3. Summary of repeated measures ANOVA on various taxa of macrobenthos.

Taxon	Variable	Treatment[1]		Date[2]		T X D[3]	
		F value	P>F	F value	P>F	F value	P>F
Hyalella	Abundance	27.88	<0.001	7.82	<0.0001	7.88	<0.0001
	Biomass	27.56	<0.001	3.15	0.01	7.30	<0.0001
Chironominae	Abundance	3.02	0.08	5.65	<0.0001	1.92	0.05
	Biomass	4.05	0.04	25.15	<0.0001	1.87	0.06
Chironomus	Abundance	3.80	0.04	6.99	<0.0001	0.76	0.67
Tanypodinae	Abundance	1.43	0.28	7.44	<0.0001	1.73	0.09
	Biomass	1.83	0.21	3.44	0.007	1.64	0.11
Physa	Abundance	0.88	0.45	21.15	<0.0001	1.71	0.09
	Biomass	1.45	0.24	12.90	<0.0001	2.12	0.03
Helisoma	Abundance	3.72	0.04	4.77	0.0006	0.73	0.69
	Biomass	3.39	0.06	5.45	0.0002	1.08	0.39
H. stagnalis	Abundance	2.98	0.08	4.88	0.0005	0.98	0.47
	Biomass	0.56	>0.50	1.85	0.11	1.06	0.40
Caenis	Abundance	0.57	>0.50	58.69	<0.0001	0.46	0.67
	Biomass	0.88	>0.50	44.45	<0.0001	0.64	0.78
Coenagrionidae	Abundance	0.46	>0.50	31.39	<0.0001	0.70	0.72
	Biomass	0.46	>0.50	39.22	<0.0001	0.67	0.74

1. 2,18 df.
2. 5,90 df.
3. Treatment level X sampling date interaction. Degrees of freedom (df)=10,90.

overt effects on *Hyalella*. Nevertheless, the mean abundance and biomass of *Hyalella* in the 5-µg/L enclosures , although consistently equal to or higher than that in the controls before treatment, was consistently lower after treatment (Fig. III-1), possibly as a result of a small effect. In contrast to the control and 5-µg/L enclosures, significant differences were recorded between the last pre- and the first post-treatment sampling dates and among post-treatment sampling dates in the 25-µg/L enclosures (Appendix IV) reflecting the carbofuran-induced decline in *Hyalella* and their late-season recovery at this concentration.

Abundance and biomass of Chironominae larvae in control enclosures were highest in early to mid-July and declined continuously through Sept. 8 (Fig. III-2). This pattern was also evident at 5-µg/L. At 25-µg/L, patterns of abundance were similar to those in the controls until Aug. 16 after which an apparent increase occurred, while biomass decreased sharply following treatment and stabilized during the post-treatment period in contrast to the pattern in the controls. Changes in abundance and biomass of Chironominae larvae over time were not similar among treatment levels as indicated by the significant interaction terms for both variables (Table III-3). For abundance, however, this was not evidently the direct result of carbofuran treatment, since no significant changes in abundance were noted between the last pre- and first post-treatment sampling dates at any of the treatment levels (Appendix IV). In contrast, the biomass of Chironominae larvae was significantly and negatively impacted by carbofuran at 25-µg/L, as indicated by its significant decline at this treatment level between the last pre- and first post-treatment sampling dates coupled with the absence of such a decline in the controls (Appendix IV). During the post-treatment phase of the study, the biomass of Chironominae larvae declined significantly in the control and 5-µg/L enclosures but not in the 25-µg/L enclosures (Appendix IV), reflecting the gradual recovery by this taxon in the 25-µg/L enclosures relative to the controls.

The presence of a statistically discernible impact of carbofuran on Chironominae larval biomass coupled with the absence of any such effect on its abundance suggests that the 25-µg/L treatment may have been more toxic to larger larvae than to smaller ones. There is some evidence which supports this contention. At the

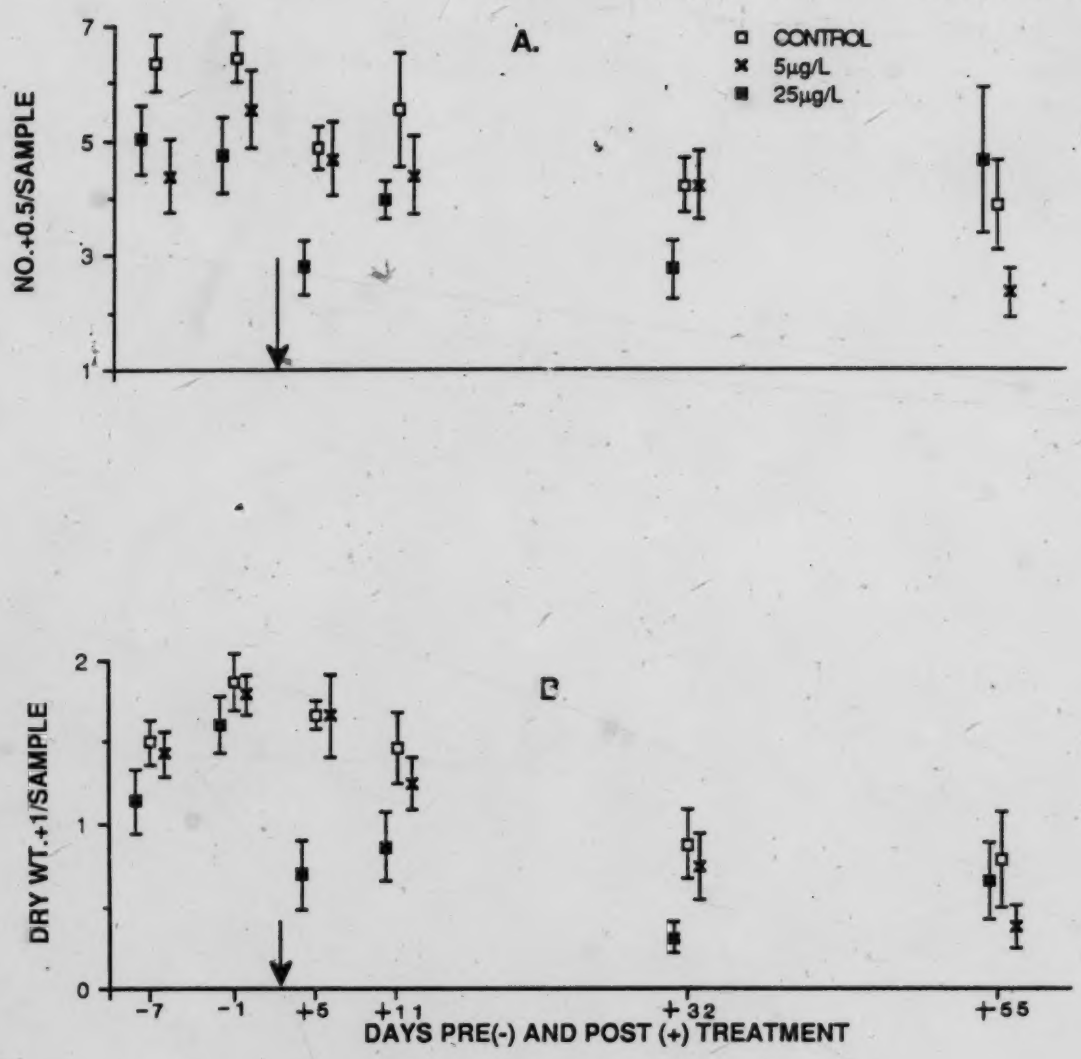

Fig. III-2. Chironominae larvae: mean (±SE) (A) abundance (square root transformed) and (B) biomass (mg) (log transformed) in control, 5µg/L, and 25µg/L enclosures (n=7 for each group) on 6 sampling dates during summer, 1987. Treatment date (July 15) indicated by arrow. Absicca co-ordinates: -7, -1, +5, +11, +32, and +55 correspond to July 8, 14, 20, 26, Aug. 16, and Sept. 8 respectively. Symbols slightly offset for clarity.

time of carbofuran application, large red midge larvae of the genus *Chironomus* comprised about 44% of all the Chironominae larvae in the samples. The remaining larvae were small and although they were not identified to genus, they were not younger stages of the large *Chironomus*. It was not possible to attribute a post-application decline in abundance of *Chironomus* to the 25-µg/L treatment because of the simultaneous decline in control and 5-µg/L enclosures as well as the large residual variation (CV=59%) (Fig. III-3). However, analysis of proportions of live and dead larvae recovered from sweep net samples taken 72-96 h after treatment revealed a highly significant effect of treatment level on the proportion of *Chironomus* which were alive (Kruskal-Wallis, T=13.36, 2df, P<0.005) (Conover,1980). Of the large *Chironomus* larvae, 100% were alive in samples taken from the control and 5-µg/L enclosures while in samples taken from the 25-µg/L enclosures, only 23% were alive (Table III-4). While no attempt was made to quantify the proportion of living and dead small larval Chironominae during the 96 h post application period, large numbers of living larvae, but no dead larvae, were observed in samples from all enclosures including the 25-µg/L treatment. Moreover, after the abundance of large *Chironomus* larvae was subtracted from that of total larval Chironominae, there was no evidence of any treatment X sampling date interaction (F=0.28, 2,18df, P>0.75) based on an analysis that incorporated the three treatment levels and the last pre- and first post-application sampling dates. The between sampling date declines in mean abundance of larval Chironominae when large *Chironomus* were excluded were slight and very similar among the three treatment levels (control enclosures: 20 to 15.5; 5 µg/L enclosures: 17 to 15.7; and 25 µg/L enclosures:12 to 7.2 individuals; mean values for the last pre-treatment and first post-treatment sampling dates respectively). This suggests that carbofuran was not as toxic to the small Chironominae larvae as it was to the large larvae of *Chironomus*.

Changes in the abundance and biomass of Tanypodinae larvae between any set of adjacent sampling dates were not significantly different among treatments (Table III-3) indicating that the 5-µg/L and 25-µg/L concentrations of carbofuran did not have a sudden, deleterious impact on this taxon. Their maximum abundance and biomass in the control enclosures was recorded on July 14, declining after that to seasonal lows by Sept. 8 (Fig. III-4). For abundance, the

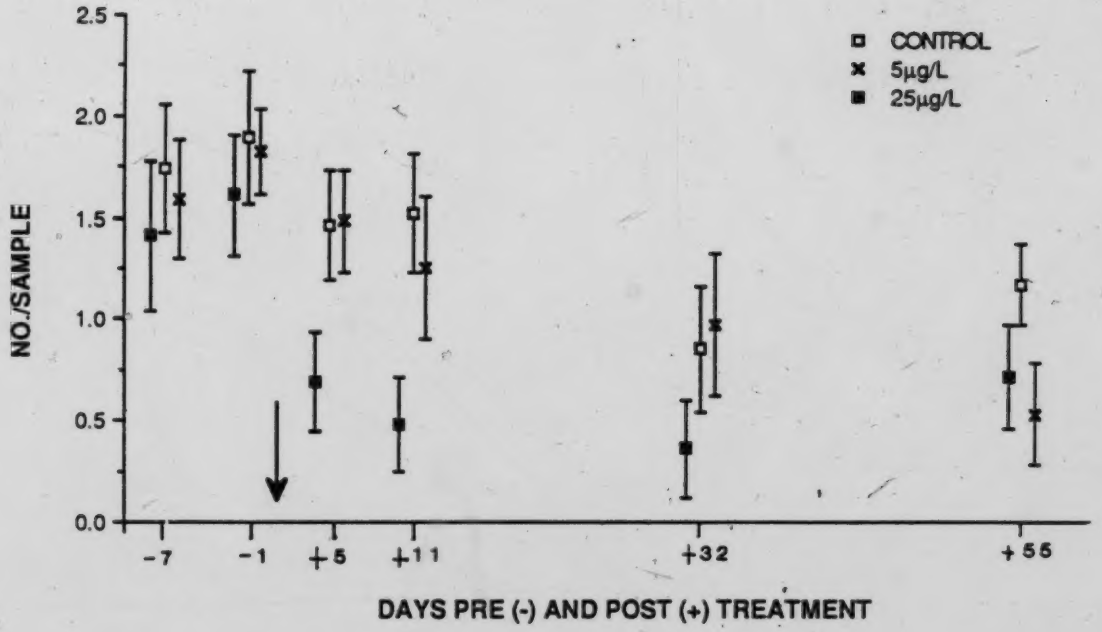

Fig. III-3. *Chironomus* larvae: mean (±SE) abundance (fourth root transformed) in control, 5µg/L, and 25µg/L enclosures (n=7 for each group) on 6 sampling dates during summer, 1987. Treatment date (July 15) indicated by arrow. Absicca coordinates: -7, -1, +5, +11, +32, and +55 correspond to July 8, 14, 20, 26, Aug. 16, and Sept. 8 respectively. Symbols slightly offset for clarity.

Table III-4. Percent of *Chironomus* larvae still alive in dip net samples taken 48-72h after treatment.

Treatment	No. Recovered[1]	%Alive[1]
Control (n=5)[2]	8.6±1.0, 10	100±0, 100
5 µg/L (n=6)[3]	7.8±1.2, 9	100±0, 100
25 µg/L (n=5)[2]	9.6±0.2, 10	23±12, 30

1. Mean±1SE, median.
2. Less than three larvae were recovered from two enclosures during the 10-min search. These two enclosures were not considered in the analysis.
3. Less than three larvae were recovered from one of the enclosures during the 10-min search. This enclosure was not considered in the analysis.

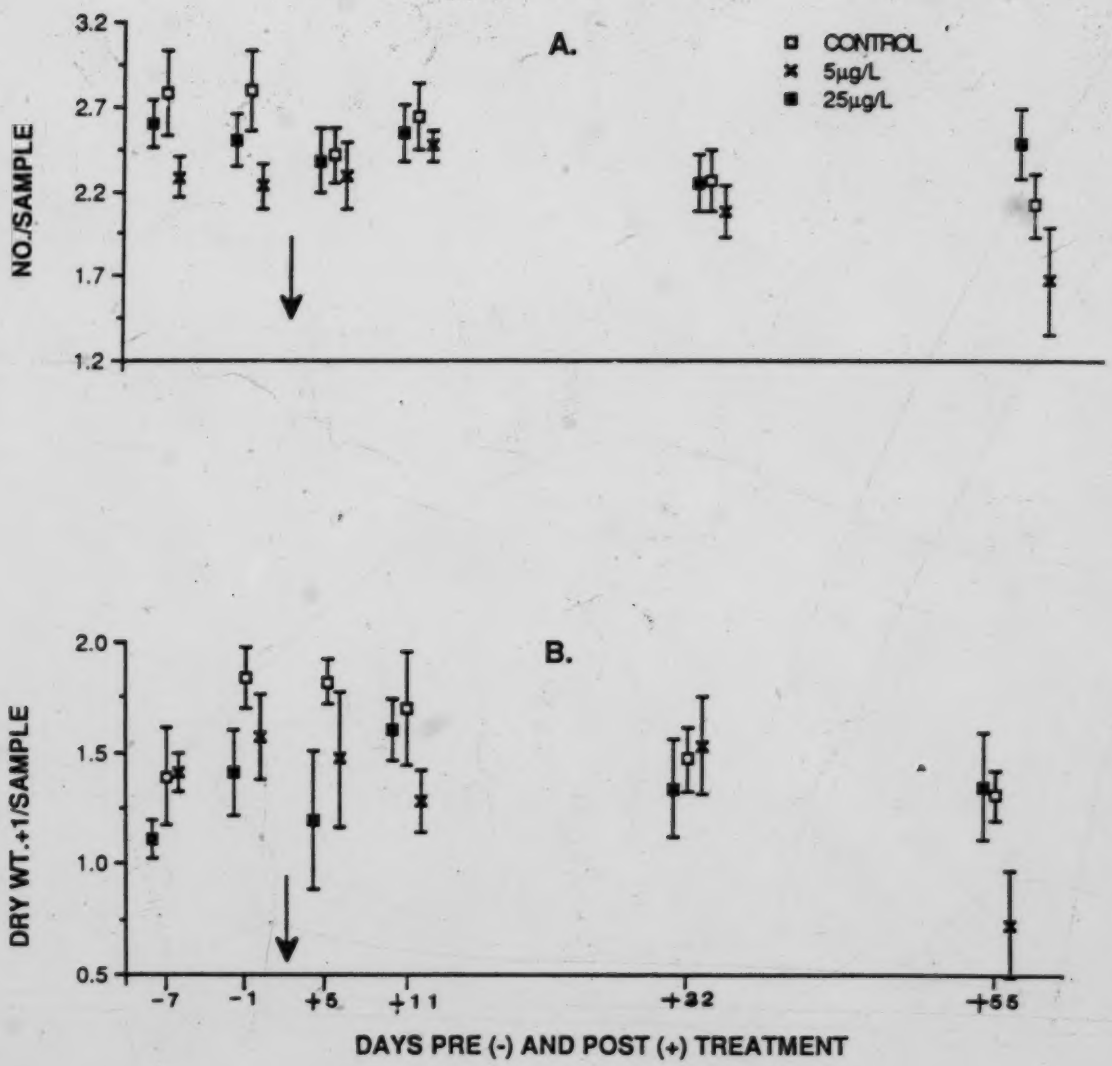

Fig. III-4. Tanypodinae larvae: mean (±SE) (A) abundance (fourth root transformed) and (B) biomass (mg) (log transformed) in control, 5µg/L, and 25µg/L enclosures (n=7 for each group) on 6 sampling dates during summer, 1987. Treatment date (July 15) indicated by arrow. Absicca co-ordinates: -7, -1, +5, +11, +32, and +55 correspond to July 8, 14, 20, 26, Aug. 16, and Sept. 8 respectively. Symbols slightly offset for clarity.

post-treatment period decline was significant while, for biomass, the apparent decline was slight but non-significant (Appendix IV). A similar pattern was recorded in the 5-µg/L enclosures for abundance and biomass, indicating the lack of any overt secondary effects on this group. In the 25-µg/L enclosures, changes in mean abundance of Tanypodinae larvae paralleled those in the control enclosures until Sept. 8 at which time it increased slightly in contrast to the significant decline in the control enclosures (Appendix IV), suggesting a possible secondary effect of the 25-µg/L concentration of carbofuran on this group. Its biomass fluctuated in the 25-µg/L enclosures, peaking on July 26 and declining slightly but non-significantly thereafter (Appendix IV) in a manner similar to the control enclosures, suggesting the absence of any strong secondary effects of this concentration on Tanypodinae larvae.

The abundance of the gastropod, *Physa*, was not adversely affected by either concentration of carbofuran as indicated by the lack of a significant interaction term (Table III-3). Nor was its biomass affected by either concentration since no significant changes in this variable were noted between the last-pre- and first post-treatment sampling dates at any of the treatment levels (Appendix IV). Both the abundance and biomass of *Physa* were relatively low in the controls during July, increased significantly between July 26 and Aug. 16 (Appendix IV) and stabilized thereafter (Fig. III-5). While there appeared to be temporal variation in the rate of increase in the abundance and biomass of *Physa* between the controls and both the 5-µg/L and 25-µg/L enclosures, the patterns of change during the post-treatment period as a whole were similar among treatment levels (Fig. III-5) (Appendix IV), suggesting the lack of any overt secondary effects of carbofuran. Nevertheless, it is noteworthy that, by Sept. 8, the abundance and biomass of this group in the 25-µg/L enclosures had surpassed those in the controls.

Neither concentration of carbofuran appeared to be directly toxic to the snail, *Helisoma*, since changes in its abundance and biomass between the final pre- and first post-treatment sampling dates were not significantly different among treatment levels (Table III-3). In the control enclosures, maximum abundance was recorded on July 26, and biomass on Aug. 16 (Fig. III-6). Between Aug. 16 and Sept. 8, abundance decreased significantly (Appendix IV)

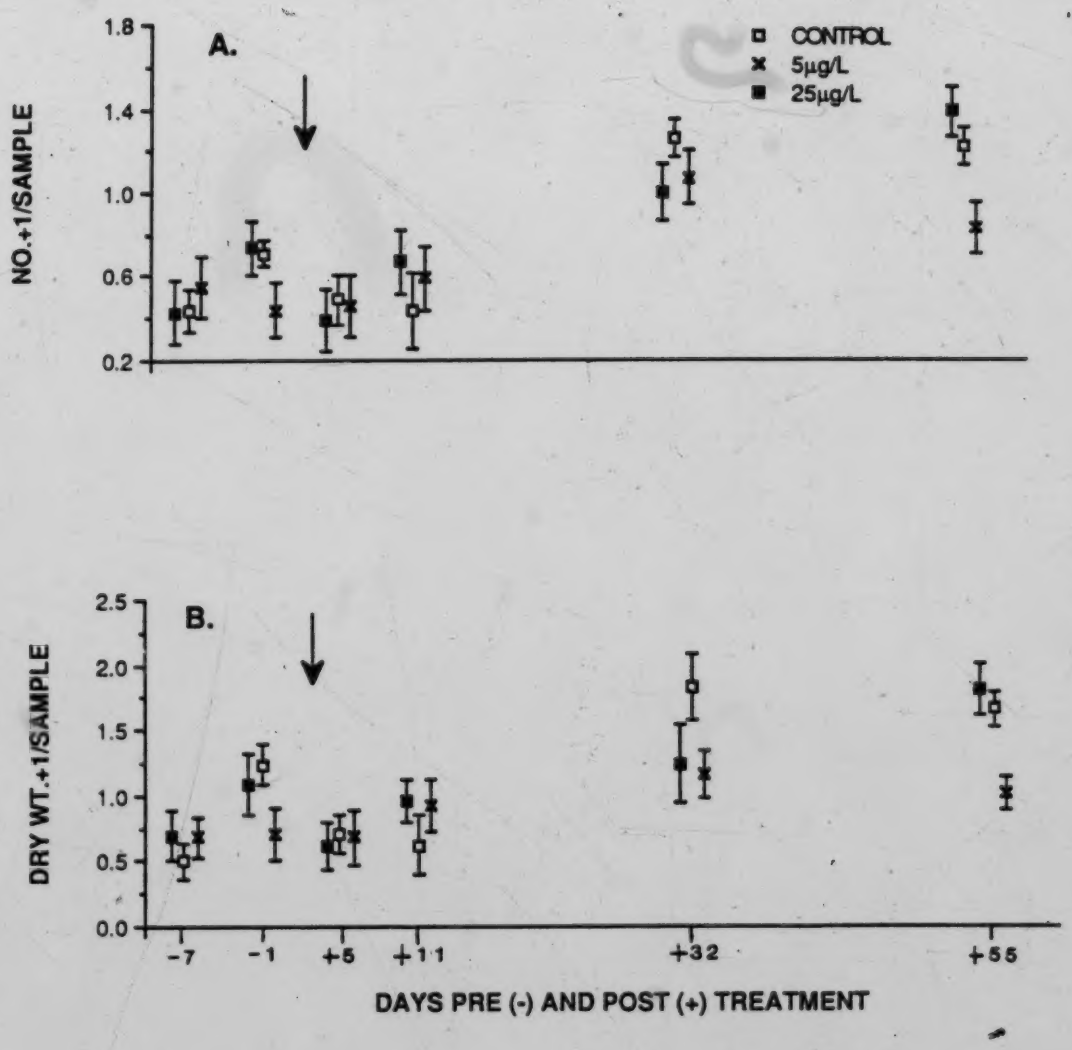

Fig. III-5. *Physa*: mean (±SE) (A) abundance (log transformed) and (B) biomass (mg) (log transformed) in control, 5µg/L, and 25µg/L enclosures (n=7 for each group) on 6 sampling dates during summer, 1987. Treatment date (July 15) indicated by arrow. Absicca co-ordinates: -7, -1, +5, +11, +32, and +55 correspond to July 8, 14, 20, 26, Aug. 16, and Sept. 8 respectively. Symbols slightly offset for clarity.

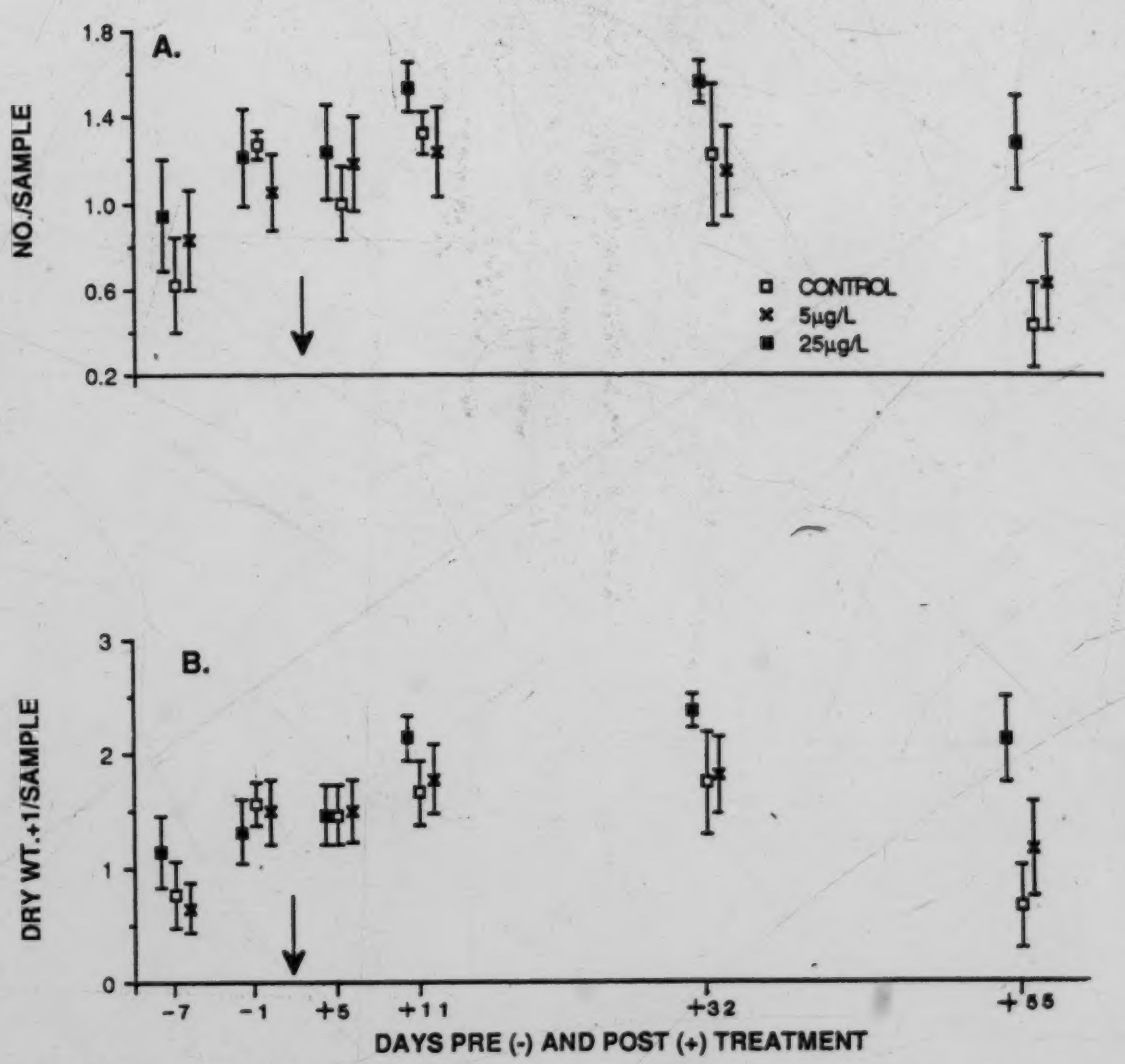

Fig. III-6. *Helisoma*: mean (±SE) (A) abundance (fourth root transformed) and (B) biomass (mg) (log transformed) in control, 5µg/L, and 25µg/L enclosures (n=7 for each group) on 6 sampling dates during summer, 1987. Treatment date (July 15) indicated by arrow. Absicca co-ordinates: -7, -1, +5, +11, +32, and +55 correspond to July 8, 14, 20, 26, Aug. 16, and Sept. 8 respectively. Symbols slightly offset for clarity.

while mean biomass also decreased, although only to a level approaching significance (P=0.10, Appendix IV). At 5 µg/L, its mean abundance declined after July 26 (Fig. III-6), although, unlike the controls, this decline was not significant (Appendix IV). The decline in mean biomass was also non-significant (Appendix IV) as was the case in the controls, suggesting that biomass probably was not secondarily affected. At 25 µg/L, patterns of change in abundance and biomass of *Helisoma* during the post-treatment period did not conform to those in the controls. Abundance and biomass peaked on Aug. 16 at higher levels than in the controls (Fig. III-6) but did not decline significantly thereafter in marked contrast to the significant and near-significant declines in abundance and biomass in the controls (Appendix IV). Abundance and biomass of *Helisoma* in the 25-µg/L enclosures exceeded that in the controls throughout the post-treatment period, the differences increasing over time and reaching maximal levels by Sept. 8.

Helobdella stagnalis was not directly affected by either concentration of carbofuran as indicated by the absence of a significant interaction effect on either its abundance or biomass (Table III-3). In the controls, mean abundance and biomass of *H. stagnalis* were fairly stable until July 26 and increased by Aug. 16, the increase in abundance being significant (Fig. III-7) (Appendix IV). Thereafter, mean abundance decreased slightly but non-significantly, while biomass remained virtually unchanged. Patterns of change in both these variables at both concentrations of carbofuran conformed to those in the controls during the post-spray period (Fig. III-7), the one exception being that no significant increases in abundance of this species occurred at either concentration during the post-treatment period (Appendix IV). The difference between the control and 5-µg/L enclosures in the magnitude of change in abundance of *H. stagnalis* during the post-treatment period was not likely a secondary effect of carbofuran, since direct effects, upon which secondary effects are contingent (Hurlbert 1975) were not detected in the 5-µg/L enclosures. Moreover, the absence of a secondary effect in the 5-µg/L enclosures coupled with the similar patterns of change in abundance and biomass between the 5-µg/L and 25-µg/L enclosures implies that secondary effects were also absent at the higher concentration.

Changes in the abundance and biomass of coenagrionid nymphs and those of the mayfly, *Caenis*, between the last pre- and first

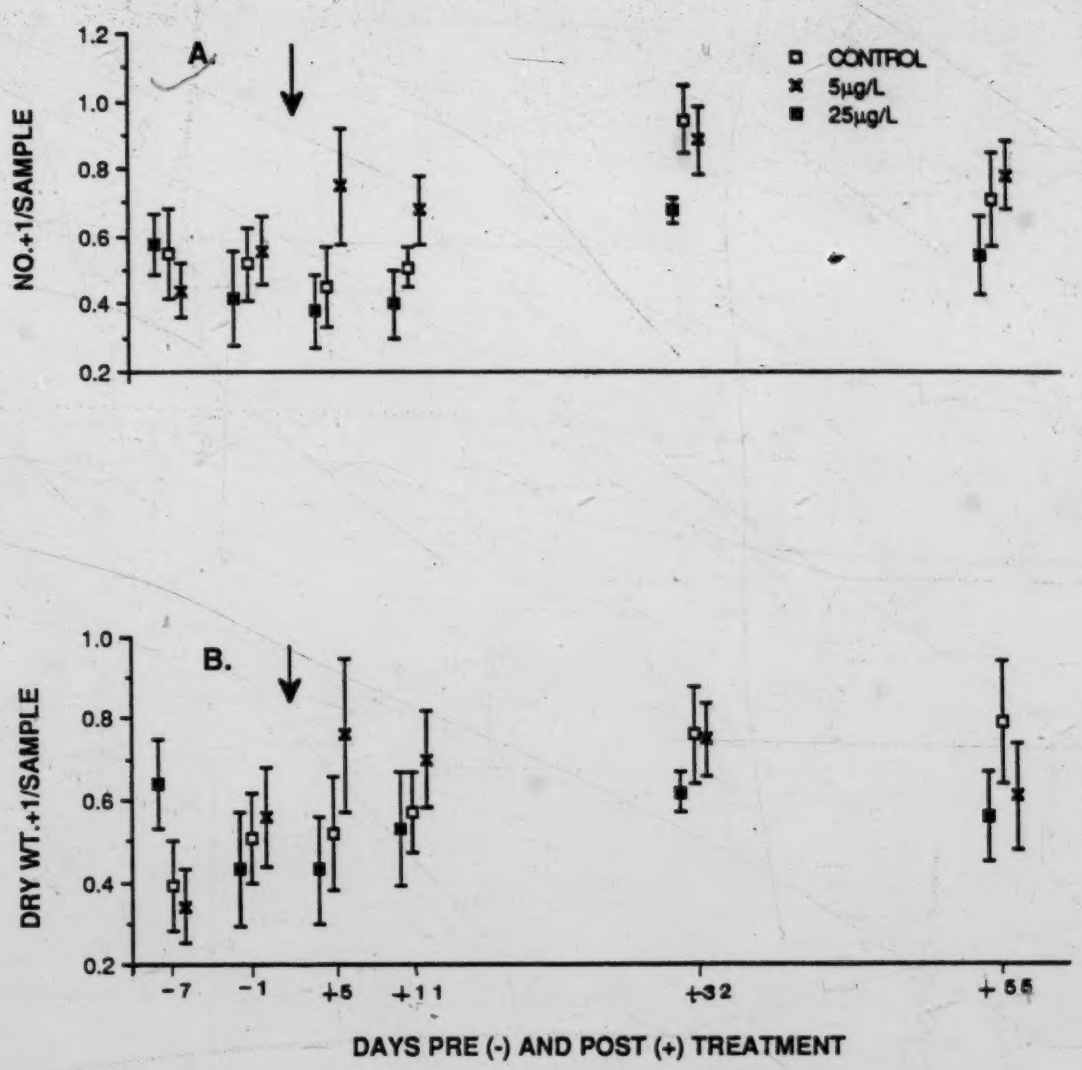

Fig. III-7. *Helobdella stagnalis*: mean (±SE) (A) abundance (log transformed) and (B) biomass (mg) (log transformed) in control, 5µg/L, and 25µg/L enclosures (n=7 for each group) on 6 sampling dates during summer, 1987. Treatment date (July 15) indicated by arrow. Absicca co-ordinates: -7, -1, +5, +11, +32, and +55 correspond to July 8, 14, 20, 26, Aug. 16, and Sept. 8 respectively. Symbols slightly offset for clarity.

post-treatment sampling dates were not significantly different among treatment levels (Table III-3). However, since abundance and biomass for both taxa were very low before treatment and on the first post-treatment sampling date at all treatment levels and since recruitment occurred mostly from late July until early September (Fig. III-8&9) after the direct effects of carbofuran were dissipated (Chapter II) , it was very unlikely that a toxic effect of carbofuran on either group would be detected. After July 20, coenagrionid abundance and biomass in the controls increased steadily through Sept. 8 (Fig.III-8), the increase in biomass being significant (Appendix IV). The abundance and biomass of *Caenis* in the controls increased significantly after July 20 (Appendix IV), peaking on Aug. 16 and stabilizing thereafter (Fig. III-9). At both concentrations of carbofuran, patterns of change in the abundance and biomass of these taxa were similar to those in the controls (Fig. III-8&9), the one exception being that the abundance of coenagrionid nymphs increased significantly during the post-treatment period at both concentrations in contrast to the pattern in the controls which was not significant (Appendix IV). The similarity among treatment levels suggests that these taxa were not secondarily affected by carbofuran at either concentration. Throughout the study, mean abundance and biomass were very similar among treatment levels for both taxa (Fig. III-8 &9), further suggesting the absence of a secondary effect.

DISCUSSION

With the possible exception of *Hyalella*, the results indicated that the 5-µg/L treatment level had no demonstrable effects on benthic invertebrates considered in this study. That the 5-µg/L concentration was responsible for the reduced post-treatment increase in *Hyalella* relative to the controls (Fig. III-1) is supported by the slightly, yet significantly higher mortality rate of *Hyalella* held in cages at this treatment level than in the controls (Chapter IV). Nevertheless, the effect of carbofuran, at 5 µg/L, on *Hyalella* within the enclosures remains open to question since changes in its abundance and biomass following treatment at this concentration were not significant, a pattern also recorded in the controls.

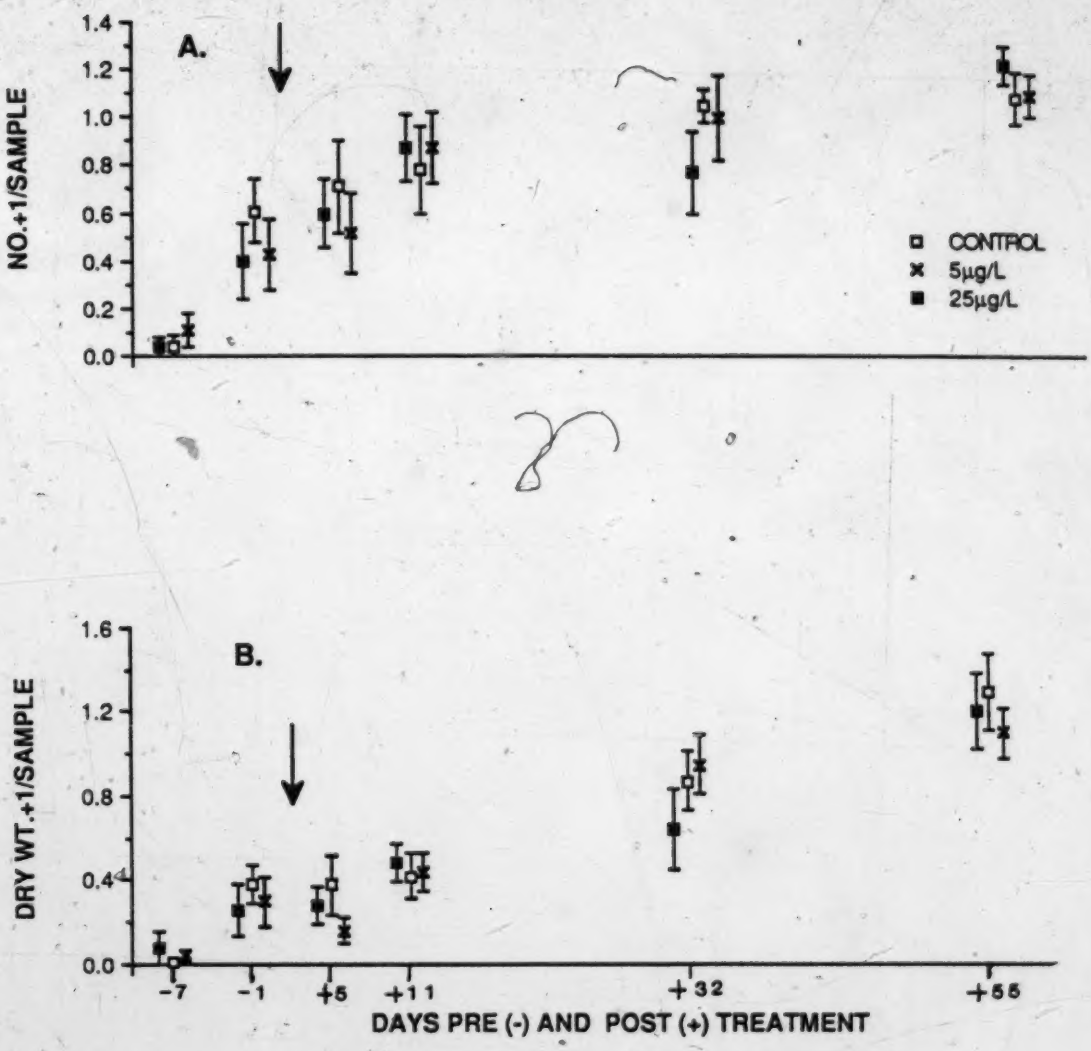

Fig. III-8. Coenagrionidae: mean (±SE) (A) abundance (log transformed) and (B) biomass (mg) (log transformed) in control, 5µg/L, and 25µg/L enclosures (n=7 for each group) on 6 sampling dates during summer, 1987. Treatment date (July 15) indicated by arrow. Absicca co-ordinates: -7, -1, +5, +11, +32, and +55 correspond to July 8, 14, 20, 26, Aug. 16, and Sept. 8 respectively. Symbols slightly offset for clarity.

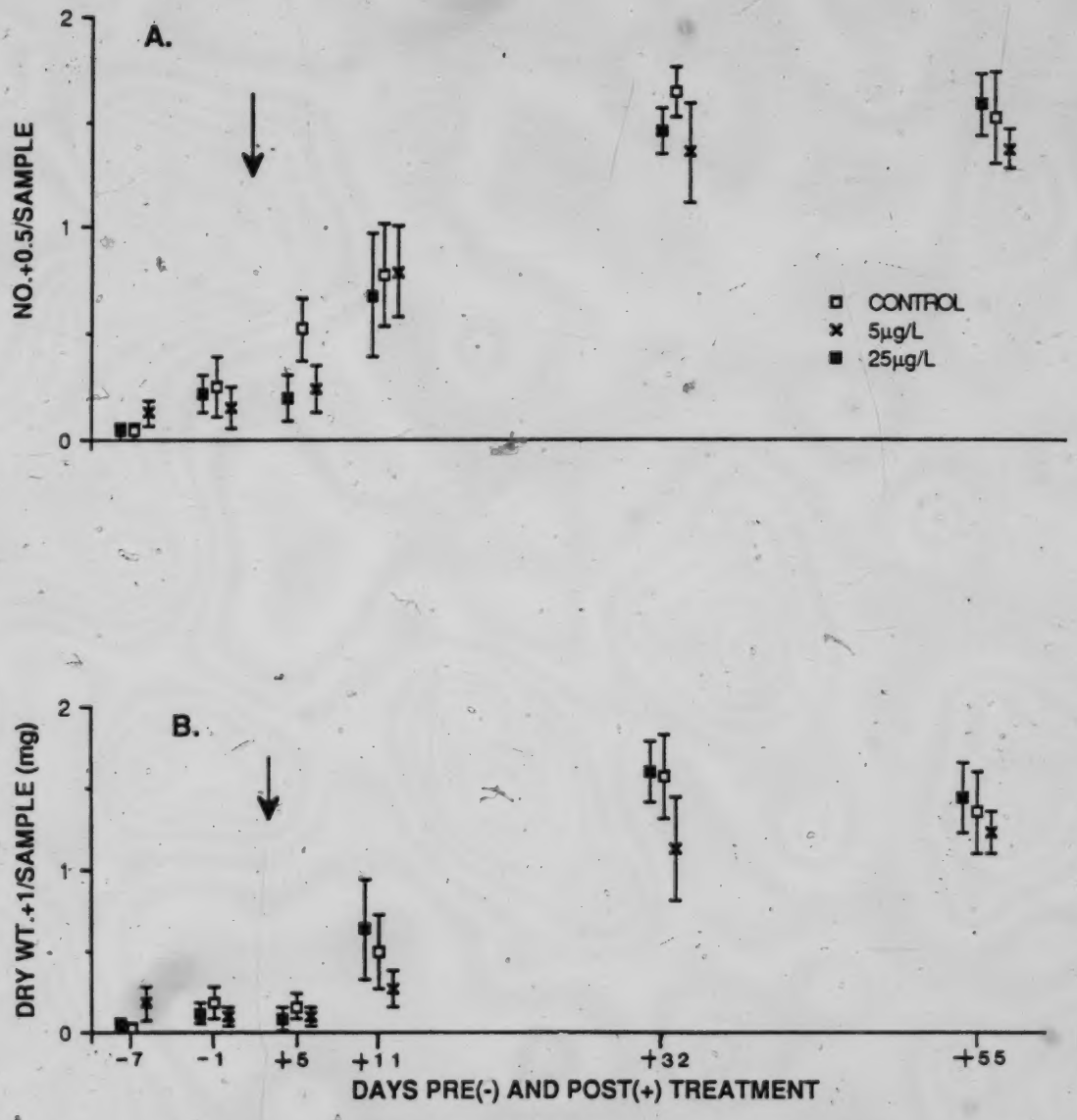

Fig. III-9. *Caenis*: mean (±SE) (A) abundance (log transformed) and (B) biomass (mg) (log transformed) in control, 5µg/L, and 25µg/L enclosures (n=7 for each group) on 6 sampling dates during summer, 1987. Treatment date (July 15) indicated by arrow. Absicca co-ordinates: -7, -1, +5, +11, +32, and +55 correspond to July 8, 14, 20, 26, Aug. 16, and Sept. 8 respectively. Symbols slightly offset for clarity.

At 25 µg/L, however, the abundance and biomass of *Hyalella* and larval biomass of the Chironominae were significantly reduced. Following application of carbofuran at 25 µg/L, mean abundance and biomass of *Hyalella* were reduced to about 10% and 6% respectively of their pre-treatment levels (Appendix IV). This reduction is consistent with and reinforces the results obtained during the multi-pond study discussed in Chapter II. Since the magnitude of the decline in mean biomass was similar to that in mean abundance, it appeared that large and small individuals, both of which are normally present during mid-summer in *Hyalella* populations (Cooper 1965, Mathias 1971) were equally susceptible to carbofuran at 25 µg/L. This implies that such a concentration is higher than the upper limit of a range of concentrations which might be expected to result in age or size-biased mortality as has been demonstrated for various crustaceans subjected to DDT (Sanders & Cope 1966, Sanders 1969).

During the post-treatment phase of the study, the abundance and biomass of *Hyalella* in the 25-µg/L enclosures increased significantly while, in the control and 5-µg/L enclosures, they remained fairly stable (Fig. III-1). This increase was in marked contrast to the results from the multi-pond study (Chapter II) in which *Hyalella* abundance did not recover following spraying but agrees with the partial recovery by *Hyalella* following its initial decimation in a pond sprayed with the carbamate insecticide, larvin (Ali & Stanley 1982); it also implies that a density-dependent mechanism could have been responsible for the different patterns of population growth exhibited by the different treatment levels. Density-dependent population growth has been demonstrated for several common macroinvertebrate species including *Hyalella* (Wilder 1940), as well as *Physa gyrina* (De Witt 1954, Brown 1979), *Lymnaea elodes* (Eisenburg 1966, 1970, Brown 1979), two other pulmonate snails (Brown 1979) and the clam *Pisidium casertanum* (Bailey & Mackie 1986). These authors suggested that fecundity was the density-dependent variable primarily responsible for the pattern of population increase recorded in their studies. Whether the surviving sexually-mature *Hyalella* in the 25-µg/L enclosures became more fecund than those in the more densely-populated control and 5-µg/L enclosures was not determined. Wilder's (1940) laboratory study, while demonstrating a doubling in fecundity when

density was reduced by 80%, also showed that growth rate and adult body size were positively related to density. Body size is, in turn, highly correlated with fecundity in *Hyalella* (Cooper 1965, Strong 1972). Thus, the inverse relationship between density and fecundity in *Hyalella* may be a two-step process in which body size is the proximate factor controlling fecundity. In populations of *Hyalella* found in lakes in latitudes approximating that of the study pond, only the overwintering females are sexually mature (Mathias 1971), and therefore, this group would have had to have grown at a relatively faster rate in the 25-µg/L enclosures than in the control and 5µg/L enclosures to bring about the density-dependent recovery. Moreover, given that *Hyalella* requires an incubation period of 8-12 days between the release of successive broods (Strong 1973) and that brood sizes range from about 4-15 individuals per clutch (Cooper 1965, Strong 1972), the relatively rapid recovery rate implies that the size differences in reproducing females must have become evident soon after the initial reduction in density. I was unable to find any information concerning growth rates of sexually mature females under conditions of different densities and unfortunately biomass data on sexually mature females alone were not collected in this study. Nevertheless, if fecundity of female *Hyalella* is more strongly related to body size than to density as the literature suggests, the relatively rapid recovery of *Hyalella* during the post-treatment phase of the study seems unlikely unless some mechanism other than fecundity was responsible. Alternatively, fecundity may not be solely size-dependent in aquatic invertebrates. Studies evaluating the reproductive output of several species of pond snails have suggested that, under certain conditions, adults may be capable of rapidly shifting the allocation of resources to reproduction (Eisenburg 1966, Brown 1979, Rollo & Hawryluk 1988). If this were also true of *Hyalella*, then females in this study could have reacted quickly to the lowered density without having to wait for a change in body size before increasing their fecundity. Finally, in assessing the recovery of *Hyalella* in the 25-µg/L enclosures the role of survival must also be considered. The laboratory study by Wilder (1940) was inconclusive regarding the effects of density on mortality rates of *Hyalella*, although it appeared that mortality rates of young amphipods were greatest at low densities while, at higher densities, they survived somewhat longer but then died rapidly, in large numbers. Moreover, with regards to populations of

semelparous, short-lived animals such as *Hyalella*, mortality is not generally believed to be density-dependent (Pianka 1970). Nevertheless, both Eisenburg (1970) and Brown (1979) believed that survivorship may have been affected negatively, at least to some extent, by density in populations of pulmonate snails which, at the latitudes studied, were also semelparous, short-lived animals. In addition, Cooper (1965) has shown that mortality rates of young *Hyalella* are high throughout the summer and that the chronology of mortality appears to be age-related such that heavy mortality within the older, juvenile portion of the population peaks during mid-summer. It is possible that mortality of older juvenile *Hyalella* in the control and 5-µg/L enclosures may have been high during the post-treatment phase of this study and, therefore, the lower rate of increase in both abundance and biomass within these enclosures may have resulted from this high mortality coupled with continued natality and subsequent growth. However, in the 25-µg/L enclosures from which juvenile *Hyalella* had been almost completely extirpated as a result of the treatment, their mortality was unlikely to have been an important factor in the post-treatment phase, and the demonstrated increase in abundance and biomass were, therefore, likely the result of natality and growth of newborn *Hyalella* alone.

The reason for the recovery of *Hyalella*, recorded in this study but not in the multi-pond study discussed in Chapter II, remains an open question. While no definitive answer can be offered, it is possible that this difference was related to differences in levels of reproductive activity in the two studies. If, as suggested in Chapter II, reproductive activity was very low during the post-treatment phase of the multi-pond study, one would expect both a decline in the control ponds associated with high mid-summer mortality of subadults as described by Cooper (1965) and a stable population size in the treated ponds since most of these subadults would have died shortly after their exposure to carbofuran. Fig. II-5A (Chapter II) provides some evidence of these trends in the shallow zones of ponds used in the multi-pond experiment. This situation contrasts with both the slow increase in *Hyalella* abundance in the control and 5-µg/L enclosures and the more rapid increase in abundance in the 25-µg/L enclosures which, as has been suggested above, infer a relatively high level of reproductive activity throughout the summer

in this study.

In the future, it will be necessary to understand, more completely, not only the influence of density on recruitment and mortality, but also how density influences fecundity, if we are to be capable of predicting recovery rates of aquatic invertebrates following short-term exposures to various toxins.

The abundance of Chironominae larvae was apparently not affected adversely in the short-term by either the 5-µg/L or 25-µg/L concentrations of carbofuran, consistent with the results recorded in the multi-pond study (Chapter II). The biomass of Chironominae larvae, however, was reduced to only 17% of its pre-treatment level following application of carbofuran at 25 µg/L (Appendix IV) and the results indicated that this was due to the relatively greater sensitivity of large *Chironomus* larvae to this pesticide than the other species of Chironominae which occurred in the study pond on the treatment date. The toxicity of carbofuran to larvae of the genus *Chironomus* is apparently highly variable, the 48-h LC50 value for *C. tentans* being 1.6 µg/L (Karnak & Collins 1974) and the 48-h EC50 value for *C. riparius* being 56.0 µg/L (Johnson 1986). In this study, I did not determine the species of *Chironomus* which was killed by the 25-µg/L treatment. Nevertheless, the high mortality, of this unknown species of *Chironomus* is consistent with the range of values reported in the literature. I was unable to find any information on the toxicity of carbofuran to other genera of larval Chironominae with the exception of *Goeldichironomus holoprasinus* which suffered an 88% decline following application of carbofuran at approximately 94 µg/L (calculated from Mulla & Khasawinah 1969). Nevertheless, the results indicate that, with the exception of the large *Chironomus* larvae, the Chironominae were tolerant of carbofuran at 25 µg/L.

During the post-treatment phase of this study, Chironominae abundance and biomass fluctuated somewhat in the 25 µg/L enclosures but did not show any consistent increase or decrease. During this period, abundance and biomass decreased in the 5 µg/L and biomass decreased in the control enclosures (Fig.III-2). These reductions were likely the result of pupation and emergence which peaked in July in the study pond (Fig.III-10) as well as a failure to repopulate the enclosures. The failure to repopulate the enclosures is not surprising given that most chironomids oviposit along shorelines and, after the eggs hatch, first instar larvae disperse

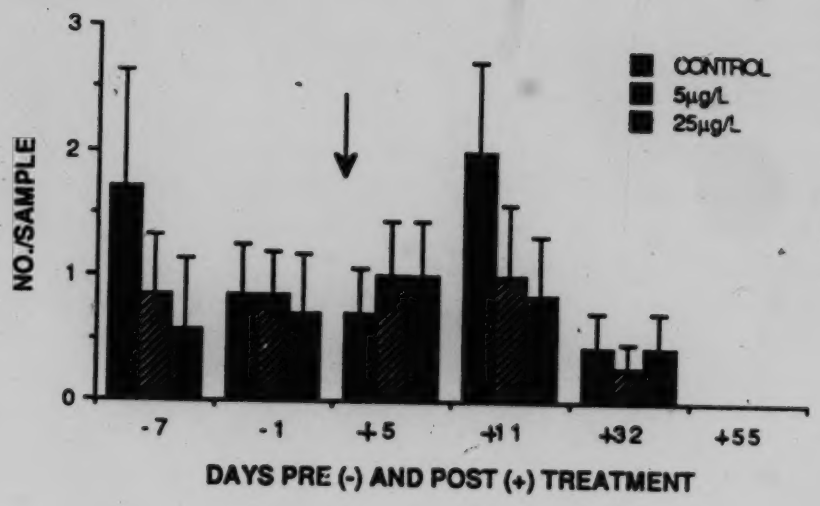

Fig. III-10. Chironomidae pupae: mean (±1SE) abundance in control, 5µg/L, and 25µg/L enclosures (n=7 for each group) on 6 sampling dates during summer, 1987. Treatment date (July 15) indicated by arrow. Abscissa co-ordinates: -7, -1, +5, +11, +32, and +55 correspond to July 8, 14, 20, 26, Aug. 16, and Sept. 8 respectively.

passively by drifting in the water column (Davies 1976). In this study, passively dispersing larvae could not enter the enclosures, thus young larvae found within enclosures in August or September would have to have originated from egg masses deposited on the water surface or on vegetation within the enclosures, a phenomenon that produced more larvae only in the 25-µg/L treated enclosures, possibly because potential competitors (e.g. *Hyalella*) or predators were reduced therein.

Tanypodinae larvae were not directly affected by carbofuran at either treatment level in this study. During the post-treatment phase their abundance decreased in the control and 5-µg/L enclosures while their biomass decreased only in the 5-µg/L enclosures. Within the 25-µg/L enclosures, neither their abundance nor biomass changed significantly during the study (Fig.III-5). Differences in abundance at the end of the study between the 25-µg/L treatment level and the other two treatment levels may or may not have been due to carbofuran-induced changes in the benthos of the 25-µg/L enclosures. Examination of several larvae suggested that the genera *Procladius* and *Ablabesmyia* dominated the Tanypodinae throughout the post-treatment period. The former is considered to be an omnivore (Armitage 1968, Roback 1969, Wrubleski & Roback 1987), while the latter is a predator (Armitage 1968, Roback 1969), making it unlikely that they would compete for food to any significant extent with herbivores such as *Hyalella* or *Chironomus*. On the other hand, the direct or indirect effect of carbofuran on potential competitors such as *Chaoborus* or prey such as copepods and cladocerans (Armitage 1968) were not evaluated in this study, and therefore the possibility of a secondary effect on this group cannot be discounted.

The two snail genera considered in this study (*Physa* and *Helisoma*) showed no susceptibility to either concentration of carbofuran (Fig.III-6&7). Other field studies examining the effects of the pyrethroid cypermethrin (Crossland 1982), the organophosphate diazinon (Arthur et al. 1983) and various chlorinated hydrocarbons (Hanson 1952) on pond macroinvertebrates have all demonstrated a high level of resistance by snails. In a laboratory study, *Helisoma* was not affected by concentrations of the pyrethroid insecticide fenvalerate which resulted in virtually 100% mortality of all the arthropods tested (Anderson 1982).

Another lab study showed that the carbamate insecticide carbaryl was not toxic to young *Lymnaea stagnalis* at concentrations below 4 ppm (Bluzat & Seuge 1979), a concentration 160X higher than the 25-µg/L concentration used in this study.

The results did not indicate any strong secondary effect of carbofuran on *Physa* during the post-treatment phase of this study (Fig. III-5). This was not surprising since snails are scrapers and, as such, should not compete for food with deposit-feeding *Hyalella* or sediment-dwelling *Chironomus* larvae (but see below). Moreover, this also implies that the decrease in *Hyalella* following exposure to 25 µg/L of carbofuran did not result in a detectable shift in predation towards physids by predators such as *Helobdella stagnalis* which is known to feed on both amphipods and snails (Davies & Everett 1975).

In contrast to *Physa*, the post-treatment abundance and biomass of *Helisoma* may have been influenced indirectly by the 25-µg/L application of carbofuran. *Helisoma* abundance and biomass in the 25-µg/L enclosures increased slightly but non-significantly during the post-treatment phase of the study, while in the control and 5-µg/L enclosures, they decreased substantially by the last sampling date (Fig. III-6). This difference between the 25-µg/L treatment level and the control and 5-µg/L levels may be the result of a release from competition with either *Hyalella* or *Chironomus* which were nearly extirpated in the 25-µg/L enclosures following application of the pesticide. However, it implies that, under normal circumstances, *Helisoma* competes with either *Hyalella*, *Chironomus* or both. While these three organisms are herbivorous, *Helisoma* like *Physa*, is a scraper associated mainly with submersed aquatic plants (Pip 1978) whereas the latter two are deposit feeders on epibenthic algae and detritus (Hall et al. 1970, Hargrave, 1970). Therefore, it does not seem likely that *Helisoma* would compete for food with either of these organisms. Nevertheless, it is noteworthy that when most aquatic insects and crustaceans were depleted in a pond treated with cypermethrin, the abundance of the snail *Lymnaea peregra* declined only to about 50% of its pre-treatment abundance over the approximately 3-month post-treatment phase of the study, whereas in a control pond in which insects and crustaceans were not depleted, *L. peregra* was reduced to a much greater extent (i.e. to about 18% of its pre-treatment numbers) (Crossland 1982). Moreover, in a laboratory

experiment, Woltering (1983) showed that under conditions favoring amphipods (i.e. low biomass of predatory fish), planorbid snail biomass declined, whereas when amphipod biomass was low, that of planorbid snails was high. He further suggested that the two organisms competed for food in his experimental aquaria. The patterns in the above studies are similar to those observed for *Helisoma* in this study and lend support to the idea that *Helisoma* was released from the effects of interspecific competition with *Hyalella*, *Chironomus* or both in the enclosures receiving 25 µg/L of carbofuran.

The mayfly nymph *Caenis* occurred in very low numbers at each treatment level prior to application of carbofuran and during the early post-treatment period and therefore it was not possible to assess the direct toxic effects of the two treatment levels on this group. Thereafter, its abundance and biomass increased steadily and by September it was a numerically important component of the total benthos (Fig. III-9). As was found in the multi-pond study of 1986, the carbofuran-induced depletion of *Hyalella* and *Chironomus* did not have any effects on this group since patterns of change in its abundance and biomass over time were not different among treatment levels. This result contrasts with a study which suggested that *Chironomus tentans* limited populations of *Caenis* either through competition or predation (Hall et al. 1970). However, the increase in *Caenis* in this study occurred over a period of time when *Chironomus* larvae were scarce at all treatment levels (Fig.III-3&9). Thus, it is not surprising that the rate of increase of *Caenis* was similar among treatment levels in this study.

As a group, the herbivores considered in this study including Chironominae larvae, *Physa* and *Helisoma*,as well as larvae of the omnivorous group, Tanypodinae, increased in abundance, biomass or both by the last sampling period in a manner which was not paralleled at the other two treatment levels (Fig.III 2, 4-6, 9). While the differences were slight in all cases except for *Helisoma*, and could be explained simply in terms of sampling variation, it is nevertheless interesting that this trend emerged for each group. Changes in the abundance or biomass of a given group of benthic invertebrates following manipulation of an ecosystem are often associated with compensatory changes in other groups, possibly the result of varying levels of interspecific competition (Kajak 1963,

Hall et al. 1970, Cantrell & McLachlan 1977, Crowder & Cooper 1982, Cuffney et al. 1984) and it is possible that the relatively greater gains in abundance or biomass of the above-mentioned herbivorous and omnivorous groups in the 25-µg/L enclosures at the end of this study may have been responses to the sharp reductions in abundance and biomass of *Hyalella* and *Chironomus* larvae which occurred after application of the pesticide. Other studies have demonstrated that, during the post-treatment period, disproportionate increases by one or more taxa of benthic invertebrates in insecticide-treated ponds sometimes follow insecticide-induced, large-scale decreases by the same or other taxa (Kennedy et al. 1970, Sanders et al. 1981).

The effects of carbofuran on two obligate predatory groups, *H. stagnalis* and Coenagrionidae were evaluated in this study. Carbofuran seemed non-toxic to *H. stagnalis* and no indirect effects were recorded on this species during the post-treatment period. Nymphs of Coenagrionidae were not abundant at the time of treatment and thus it was not possible to evaluate the direct effects of carbofuran on this group. However, over the whole season, the patterns of change in the abundance and biomass of members of this family were similar in all treatment levels (Fig.III-8) suggesting that it was not secondarily affected in any way. These observations agree generally with those of the 1986 multi-pond study (Chapter II) and suggest that prey abundance and biomass were not altered sufficiently by carbofuran to cause a change in the abundance and biomass of these predators. This result is not surprising in view of the demonstrated tendency of predators such as odonates to switch prey following alterations in relative densities of various prey species (Akre & Johnson 1979, Wallace et al. 1987), and is in keeping with the opinion that habitat complexity may influence predator densities to a greater extent than the abundance of prey alone (Benke 1976, Crowder & Cooper 1982, Folsom & Collins 1984, Wallace et al. 1987).

In order to understand more fully the secondary or indirect effects of insecticides on benthic invertebrate assemblages, future research must examine, among other things, the role of varying densities of normally abundant taxa which may be particularly sensitive to an insecticide, on densities of other potentially less-sensitive taxa over long periods of time.

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CHAPTER IV

TOXICITY OF CARBOFURAN TO SELECTED MACROINVERTEBRATES IN PRAIRIE PONDS

INTRODUCTION.

Toxicity studies conducted in the laboratory may not always accurately predict the effects of pesticides in particular natural freshwater systems (Crossland & Elgar 1983) partially because pesticide bioavailability is strongly influenced by environmental factors (Nimmo 1985, Spacie & Hamelink 1985) and, because ecological stresses may affect organism sensitivity (Chapman 1983). Pond or lake studies are used to validate predictions of the effects of chemicals in particular freshwater systems (Crossland & Stephenson 1979). However, studies of aquatic ecosystems which involve sampling populations of patchily-distributed macroinvertebrates are problematic in that separating organism response from sampling variability is often difficult (Hodson & Millard 1978). Another potential problem is that certain uncommon organisms which are, nevertheless, considered by the researcher to be important, may be difficult to sample. A compromise between the laboratory and pond or lake study approaches involves the assessment of the *in-situ* toxicity of pesticides to selected groups of animals by confining them in cages in ponds which are to be treated with pesticides. Such an approach has been used to examine pesticide toxicity to and/or uptake by several species of fish (Hurlbert et al. 1970, Kingsbury 1976, Kingsbury & Kruetzweiser 1987). Disadvantages of this approach are that 1) replication of a number of pesticide concentrations, sufficient for the determination of LC50's, is usually not feasible in pond or lake studies; 2) the time required to collect or rear a sufficient number of individuals for testing limits the number of species that can be studied; 3) confining animals in small cages for the purpose of assessing mortality precludes the study of many secondary effects (*sensu* Hurlbert 1975); and 4) confining certain species in single-species cultures may lead to cannibalism. The advantages of this technique are that 1) it is possible to assess mortality for a given

concentration of a pesticide under naturally-occurring environmental conditions and 2) it is possible to select which species to study.

In this study, I examined the toxicity of carbofuran to selected taxa of benthic macroinvertebrates in prairie ponds during the summers of 1986 and 1987. My objectives were 1) to validate the results obtained in Chapter II and III, specifically to consider the effects of carbofuran on the following taxa: the amphipod, *Hyalella*, the midge, *Chironomus tentans*, damselfly nymphs of the genus, *Enallagma*, and caddisfly larvae of the genus *Limnephilus*,and, 2) to examine the toxicity of carbofuran to *Gammarus lacustris*, a species not sufficiently common in samples collected from the study ponds to warrant analysis (Chapters II and III).

STUDY AREA AND METHODS.

1986: The study area and ponds were those described in Chapter II.

During the two-day period before spraying, adult *G. lacustris* and late-instar larvae of *Chironomus tentans* were collected from Wakamao Lake (54°14' N, 113°34' W) about 7 km west of the village of Clyde in central Alberta. To confirm the species identification for *C. tentans*, several larvae were reared in the laboratory until they emerged. All adults were subsequently identified as *C. tentans*. Similar-sized nymphs of the genus *Enallagma* (Coenagrionidae: Zygoptera) were collected from a dugout, also located near Clyde. These invertebrates were kept in water-filled buckets which were placed in coolers over bags of ice. On July 23, eight *G. lacustris*, six *C. tentans*, or six *Enallagma* were caged in a series of 500-mL plastic containers, one taxon per container. Mosquito netting (mesh size = 1 mm) was placed over the top of each container with an elastic band to hold it in place. For each taxon, six containers were placed on the bottom of each pond at randomly selected shallow sites (between 25 and 75 cm deep), another six were placed at randomly-selected deep sites (76 to 125 cm deep) of two control and two carbofuran-treated ponds. Each cup contained a small amount of sediment and aquatic macrophytes collected from the respective

ponds. Following placement of the cages (July 23), the two treated ponds were sprayed the same day with carbofuran according to techniques described in Chapter II. The initial target concentration was 14 µg/L, a concentration that might be expected in a pond with a mean depth of 1 m following an incidental contamination from a direct overflight while spraying for grasshoppers at the recommended rate. This process, including the placement of another series of containers containing the above taxa, was repeated on July 30 using two other treatment and control ponds.

The containers were retrieved 72-96 h after treatment from each pond and their contents were gently washed through a 500-µm sieve, a procedure that permitted the counting of live and dead organisms. Dead organisms included those that were still whole but no longer responded to touch as well as those which were fragmented but whose remains (usually head capsules) were identifiable. Of the original number of organisms placed in each container, the proportion remaining alive and the proportion found dead were calculated. For each of the six cages per taxon in each depth zone in each pond, averages of these proportions were also calculated.

Data were arcsine transformed prior to analysis (Sokal & Rohlf 1981). A split-plot design (Steele & Torrie 1960, p.232) incorporating the main plot effect of treatment and the subplot effects of depth and treatment-depth interaction was used to evaluate the effects of carbofuran at the two depths on the proportions remaining alive and found dead for each of the macroinvertebrates tested. When significant interaction effects were found, the simple effects were analyzed (Steele & Torrie 1960, p.202) using protected LSD tests (Snedecor & Cochran 1980, p.234). Methods for obtaining the appropriate standard errors and, where applicable, weighted t-values are outlined by Steele & Torrie (1960, p. 235-236).

1987: Procedures for collecting the invertebrates were similar to those in 1986 with the exception that adult and nearly full-grown *Hyalella* and late-instar larvae of the genus *Limnephilus* (Limnephilidae: Trichoptera) were used in place of *G. lacustris* and *Enallagma*. *C. tentans* larvae were tested again, and species identification was confirmed as in 1986.

Invertebrates were placed in buckets of aerated pond water and kept at about 20°C. On July 15, ten *Hyalella*, ten *C. tentans* larvae or eight *Limnephilus* larvae were placed in cages, one taxon per cage, as described above. For each taxon, cages were randomly assigned to 21 polyethylene enclosures, one cage per taxon per enclosure. The enclosures were described in Chapter III. Seven enclosures were designated as controls (receiving no carbofuran), seven as 5-µg/L and seven as 25-µg/L enclosures. Immediately after placement of the cages, the enclosures were treated with the appropriate amount of carbofuran in a manner described in Chapter III.

The cages were retrieved from 72-96 h after treatment and the numbers of living and dead animals in each cage were recorded. Criteria for distinguishing dead organisms were as described in the 1986 study. Of the original number of organisms placed in each cage, the proportion which remained alive or were found dead were determined. A Kruskal-Wallis test (Conover 1980, p.229) was used to determine whether there were differences among treatment levels in the median proportions remaining alive or found dead for each taxon. Where significant differences were found in the overall analysis, multiple comparisons procedures (Conover 1980, p.231) were used to compare each of the carbofuran concentrations with the control.

RESULTS

1986: Concentrations of carbofuran in the treatment ponds 16 h after spraying were 9, 14, 32 and 32 µg/L in ponds T1, T2, T3 and T4 respectively.

Three of the 288 cages were partially broken upon retrieval. Data from these cages were not used in determining average proportions remaining alive or found dead. Of the original numbers of animals placed in the cages, the mean percentages of *G. lacustris, C. tentans* and *Enallagma* which were recovered, either alive or dead, at the end of the study were 93, 68 and 85% respectively. I assumed that animals not recovered at the end of the study had either decomposed beyond recognition or had been cannibalized or scavenged by others in the cage because remnants of individuals were found in the containers. There was no

evidence that they could have escaped.

Since the experiment began on July 23 for four ponds and on July 30 for the other four, the effect of date was initially included in the analysis. Neither date nor its interaction with treatment were significant for any of the taxa ($P>0.4$, 1,4df, in all cases). Therefore, ponds were pooled across dates and the analyses repeated.

The effect of carbofuran on the proportions of *G. lacustris* remaining alive or found dead varied with depth as indicated by the significant interactions between treatment level and depth for both variables (Table IV-1). Analysis of the simple effects indicated that, in the shallow zones, the proportion surviving was significantly reduced and the proportion found dead was significantly higher in treated ponds than in controls (Table IV-2). In treated ponds, the proportion of individuals which survived was significantly lower, and the proportion found dead, significantly higher, in the shallow zone than in the deep zone (Table IV-2).

For *C. tentans*, the effect of carbofuran on the proportion of larvae surviving varied with depth as indicated by the significant interaction term for this variable, while the proportion of larvae found dead was affected uniformly and positively by carbofuran across depths (Table IV-1). Carbofuran significantly reduced the proportion of *C. tentans* larvae which survived in both the shallow and deep zones of treated ponds when compared to control ponds. However, the magnitude of the reduction was greater in the shallow zones (Table IV-2). Survival did not differ significantly between depths within either carbofuran-treated or control ponds, although, in treated ponds, it was somewhat higher in deep than in shallow zones (Table IV-2). The proportion of *C. tentans* larvae found dead was higher in carbofuran treated ponds (0.52) than in control ponds (0.09).

For *Enallagma* nymphs, there were no significant differences in the proportions remaining alive or found dead (Table IV-1), suggesting that carbofuran was not overtly toxic to this taxon within the range of concentrations in the ponds after spraying. Nevertheless, the mean proportion surviving was lower and the mean proportion found dead, higher, in the treated ponds than in the control ponds (Table IV-2), suggesting that some carbofuran-induced mortality may have occurred.

Table IV-1. Analysis of the effect of carbofuran on selected taxa of macroinvertebrates held in cages at different depths in the study ponds in 1986.

Taxon	Variable	Treatment[1]		Treatment X Depth[1]	
		E	P>E	E	P>E
G. lacustris	Alive[2]	11.8	0.014	10.3	0.018
	Dead[3]	9.7	0.021	15.3	0.018
C. tentans	Alive	65.3	0.0002	7.0	0.04
	Dead	28.0	0.002	1.8	0.23
Enallagma	Alive	2.9	0.14	0.7	0.43
	Dead	3.8	0.10	0.9	0.38

1. 1,6df.
2. Arcsine(Sqrt(no. animals alive + original no. placed in cages))
3. Arcsine (Sqrt(no. dead animals found + original no. placed in cages))

Note: Sqrt=square root.

Table IV-2. Mean percentage of animals surviving or found dead (95% CL) after 72-96 h of exposure in cages placed in shallow and deep zones of control and carbofuran-treated ponds.[1]

Taxon	Depth	%Alive		%Dead	
		Control	Treated	Control	Treated
G. lacustris	shallow	87Aa (61-99)	12Ba (9-49)	6Aa (2-22)	77Ba (38-100)
	deep	88Aa (79-95)	64Ab (5-98)	9Aa (0-29)	30Ab (4-87)
	overall	87 (76-97)	38 (0-87)	7 (0-17)	54 (7-94)
C. tentans	shallow	73Aa (53-90)	6Ba (1-14)	6 (2-22)	51 (40-62)
	deep	53Aa (40-65)	20Ba (0-51)	13 (2-25)	54 (19-87)
	overall	62 (46-79)	13 (1-27)	9 (0-20)	52 (35-70)
Enallagma	shallow	63 (51-75)	44 (13-78)	15 (5-27)	42 (7-80)
	deep	70 (50-88)	42 (1-91)	23 (14-32)	42 (9-53)
	overall	67 (55-78)	43 (31-55)	18 (9-29)	42 (17-66)

1. Means have been backtransformed from Arcsine(Sqrt(Proportion)), where Sqrt=square-root.
For each taxon within a given depth, means of treated and control ponds which are followed by similar upper case letters are not significantly different ($P>0.05$).
For each taxon within a treatment level, means of shallow and deep zones which are followed by similar lower-case letters are not significantly different ($P>0.05$).
Simple effects comparisons have been made only where the main ANOVA (Table IV-1) indicated a significant Treatment X Depth interaction ($P<0.05$).

1987: Of the animals originally placed in the cages, the mean percentages recovered, either living or dead, at the end of the study were 91, 79 and 90% for *Hyalella, C. tentans* and *Limnephilus* respectively.

Significant differences were found among treatment levels for all three taxa (Kruskal-Wallis: $P<0.002$ in all cases). For *Hyalella*, the proportion of animals remaining alive and found dead were significantly different ($P<0.01$) in both the 5- and 25-µg/L enclosures when compared with the controls. Median survival was only slightly reduced and the median proportion found dead only slightly higher in the 5-µg/L enclosures, while in the 25-µg/L enclosures the effects were more dramatic (Table IV-3). For *C. tentans*, survival was significantly reduced ($P<0.01$) only in the 25-µg/L enclosures when compared with the controls. However, the proportion found dead was significantly higher ($P<0.01$) in both the 5- and 25-µg/L enclosures than in the controls. The median percentages of larvae surviving were similar in the control (80%) and 5-µg/L enclosures (70%), while, in the 25-µg/L enclosures, it was much lower (30%) (Table IV-3). The median proportions found dead were 0, 10 and 70% in the control, 5- and 25-µg/L enclosures respectively (Table IV-3). For *Limnephilus*, the proportions of larvae surviving or found dead were significantly affected by carbofuran only in the 25-µg/L enclosures ($P<0.01$). Median proportions surviving were 79, 73 and 0% while median proportions found dead were 0, 14 and 100% in the control, 5 and 25 µg/L enclosures respectively.

DISCUSSION

General. In assessing the toxicity of carbofuran to the macroinvertebrates examined in this study, two variables were considered; the proportion of the original numbers of animals which survived and the proportion which were found dead. When using these variables to evaluate the direct toxicity of carbofuran, an implicit assumption is that natural mortality was equal in treated and control ponds and, therefore, any increase in mortality in treated ponds was due to the toxic effect of carbofuran alone. However, predator-prey relations may be

Table IV-3. Percentage (mean±1SE, median, n=7) of animals remaining alive or found dead in cages, 72-96 h after enclosures were treated with carbofuran in 1987.

Species	Variable	Treatment Level		
		Control	5 µg/L	25 µg/L
Hyalella	%Alive	92.8±2.9, 90	67.1±8.4, 70	11.4±7.0, 0
	%Dead	4.3±2.0, 0	24.3±7.2, 20	72.9±6.0, 80
C. tentans	%Alive	72.9±6.4, 80	72.9±4.2, 70	27.1±6.8, 30
	%Dead	0.0±0.0, 0	7.1±1.9, 10	57.1±7.1, 70
Limnephilus[1]	%Alive	79.0±4.5, 75	72.9±6.4, 71	0.0±0.0, 0
	%Dead	5.9±2.8, 0	16.4±5.3, 14	94.9±2.4, 100

1. Of the original numbers of animals placed in the cages, 8.3% had pupated by the end of the test period. Since it was difficult to determine whether pupae were alive or dead, they were not included in the analysis.

altered in the presence of a contaminant in such a way as either to increase prey susceptibility or decrease prey consumption (Rand 1985) and predator efficiency (Woin & Larsson 1987). Both *G. lacustris* (de March 1981) and various species of tube-dwelling chironomids (Mulla & Khasawinah 1969) tend to be cannibalistic on weaker individuals when confined in single species cultures in the laboratory. Because of the predatory nature of *Enallagma*, it would seem possible that it, too, may cannibalize weakened individuals. Thus, the possibility cannot be overlooked that the proportion surviving and the proportion found dead may have been influenced to some degree by carbofuran-induced alterations in rates of cannibalism and/or scavenging within the containers. The proportion which survived may underestimate the toxic effect of carbofuran if cannibalism was inhibited by exposure to carbofuran and would overestimate its effect if cannibalism was enhanced by carbofuran. Conversely, the proportion found dead may overestimate the direct toxic effect of carbofuran if cannibalism on weak and dying animals was inhibited by exposure to carbofuran and would underestimate its effect if cannibalism was enhanced by carbofuran. Thus, if carbofuran modified cannibalism within the cages to some extent, overestimates of its toxic effects associated with evaluating one variable would be complimented by underestimates of its effects associated with evaluating the other variable. Therefore, a conservative measure of the toxic effect of carbofuran on a given taxon would result if both variables were significantly affected. However, caution would be advised in interpreting a significant effect on one variable only.

1986: For *G. lacustris* and *C. tentans* the proportion of animals surviving as well as the proportion found dead were significantly affected by carbofuran (Table IV-1) indicating that carbofuran was toxic to these taxa within the range of concentrations in the study ponds. I am unaware of any published information on the acute toxicity of carbofuran to *G. lacustris*. The LC50 of technical-grade carbofuran to *C. tentans* was estimated to be 1.6 ppb (or µg/L) (Karnak & Collins 1974). However, in this study, survival of *C. tentans* in the deep zones of ponds T1 and T2 in which carbofuran concentrations after 16 h were 9 and 14 µg/L respectively was similar to that in the deep zones of control

ponds (Fig. IV-1&2), suggesting that the toxicity of carbofuran to *C. tentans* in this study was slightly lower than was expected on the basis of the above-mentioned LC50. For *Enallagma*, the absence of a significant effect on survival or on the proportion of animals found dead suggests that this taxon was not susceptible to the range of concentrations found in the study ponds, although the mean proportion surviving was reduced and the proportion found dead, higher, in the treated ponds than in the controls (Table IV-2).

The effect of carbofuran on the three taxa was highly variable among treatment ponds (CV's for proportions surviving and found dead in treatment ponds were respectively, 91 and 59% for *G. lacustris*, 57 and 20% for *C. tentans*, and 39 and 34% for *Enallagma*) probably, in part, the result of the different concentrations in these ponds (Fig. IV-1&2). Survival was low and the proportion found dead, high, for all three taxa in pond T4 in which the initial concentration of carbofuran was tied for highest (32 µg/L) and degradation rate lowest (Fig. IV-1&2). In pond T3, in which the concentration of carbofuran was initially high (32 µg/L), but degradation rapid, *Enallagma* appeared to be unaffected, *G. lacustris* was affected only in the shallow zones, and *C. tentans* was affected in both the shallow and deep zones in a manner similar to that seen in pond T4 (Fig. IV-1&2). In pond T2, in which the initial concentration of carbofuran in the water was second lowest (14 µg/L), there was no apparent affect on survival or the proportion of animals found dead for *G. lacustris* and *Enallagma*, while *C. tentans* was as strongly affected in this pond as it was in ponds T4 and T3 (Fig. IV-1&2). In pond T1, in which the initial concentration of carbofuran in the water was lowest (9 µg/L), the response of *C. tentans* was as expected, namely survival of larvae was highest and the proportion found dead was lowest relative to the other three treatment ponds. However, for both *G. lacustris* and *Enallagma*, survival was relatively low and the proportion of animals found dead, relatively high, in pond T1, suggesting that factors other than the concentration of carbofuran in the water, may have increased the susceptibility of these two taxa in this pond. It is possible that the higher mortality in pond T1 was related to higher concentrations and more uniform distributions of carbofuran in

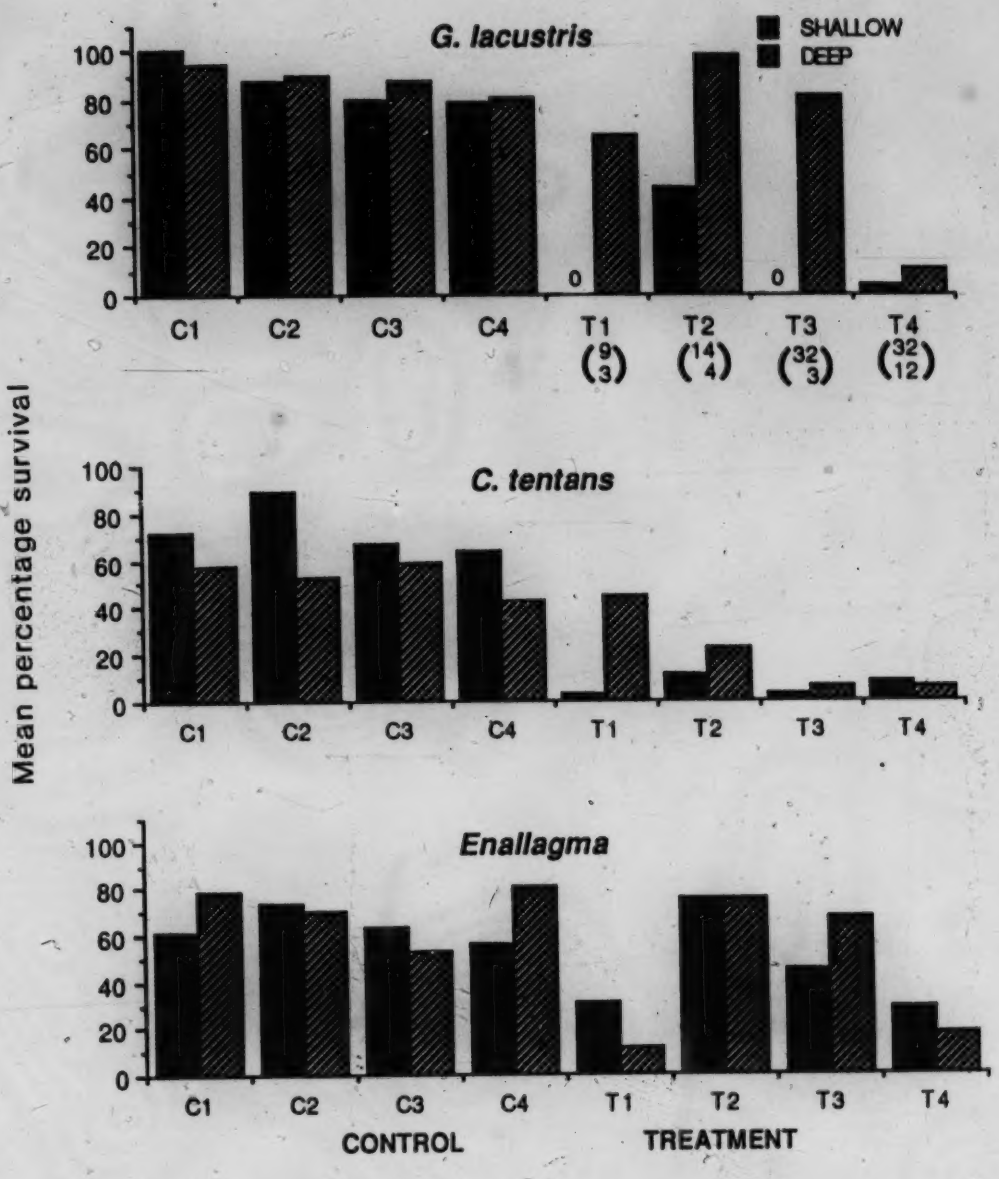

Fig. IV-1. Mean percentage of caged individuals (n=6 cages) of three macroinvertebrate taxa remaining alive after being caged for 72-96 h at shallow and deep sites in each of the four control and four carbofuran-treated ponds. Numbers in parentheses are mean concentrations of carbofuran (µg/L) in each treatment pond 16 (upper) and 124 h (lower) after spraying.

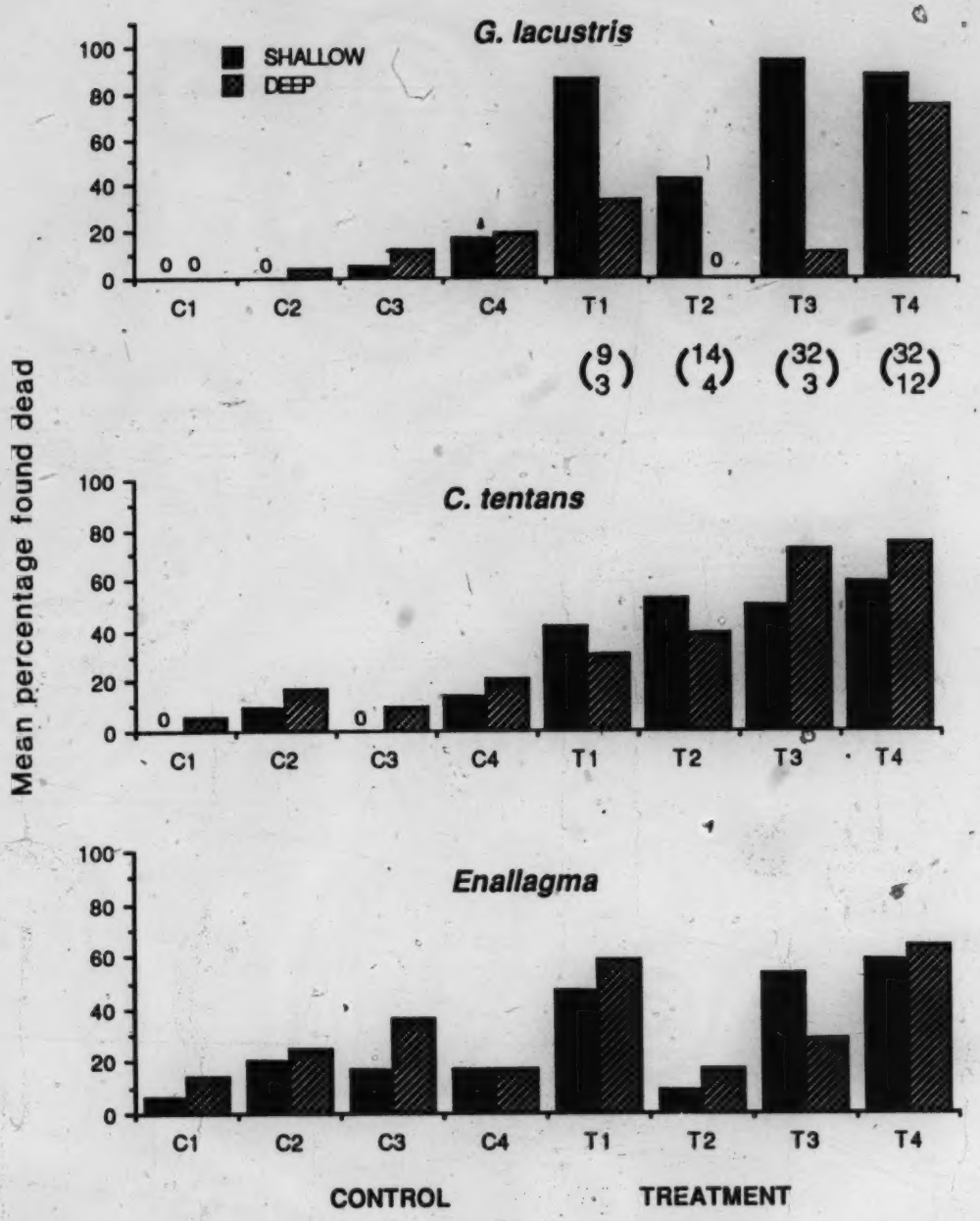

Fig. IV-2. Mean percentage of caged individuals (n=6 cages) of three macroinvertebrate taxa found dead after being caged for 72-96 h at shallow and deep sites in each of the four control and four carbofuran-treated ponds. Numbers in parentheses are mean concentrations of carbofuran (µg/L) in each treatment pond 16 (upper) and 124 h (lower) after spraying.

the sediments of this pond (mean=0.04 µg/g) than in those of the others (Chapter II). Adsorption and desorption between sediments and water may affect the uptake of pollutants by sediment-exposed animals (Lynch & Johnson 1982, Muir et al. 1985). Thus, differences in sediment concentrations of carbofuran in this study may have affected the degree of exposure of the caged animals to this insecticide. The failure of *C. tentans* to respond in a similar manner to *G. lacustris* and *Enallagma* in pond T1 is perplexing, if indeed, the toxic response of the latter two taxa was, in part, sediment-mediated. Other variables, such as pH, suspended particulate matter and macrophyte standing crop, although not evaluated in this study, may also have influenced the bioavailability and toxicity of carbofuran to the caged animals.

For *G. lacustris*, carbofuran decreased survival and increased the proportion of animals found dead only in the shallow zones, while *C. tentans* larvae were affected in both shallow and deep zones, but to a greater extent in the shallow zones (Table IV-2). These results imply that depth, or some correlate of depth, may influence the toxicity of carbofuran to certain benthic macroinvertebrates within a given range of concentrations. This result was unexpected for two reasons: first, since carbofuran is highly water-soluble (415 ppm) (Kenaga & Goring 1980), it was expected that it would disperse rapidly throughout the water column as was the case for carbaryl, another water-soluble carbamate insecticide (Gibbs et al. 1984); secondly, the low affinity of carbofuran for sediments (Klaassen & Kadoum 1979, this study, Chapter II) implies that variability in sediment characteristics which may be correlated with depth within ponds should not have a strong effect on bioavailability of carbofuran to benthic macroinvertebrates. Nevertheless, it may be noteworthy that carbofuran was detected in at least one sediment sample from shallow sites in three of the four treatment ponds while, in deep sites, carbofuran was detected in the sediments of only one pond. In this study, differences between depths in the survival and proportion of *G. lacustris* found dead may have been influenced by the different concentrations of carbofuran in the treatment ponds. These differences were greatest in ponds T1 and T2 in which initial carbofuran levels were lowest as well as in pond T3 in which the

initial concentration was high, but degradation rapid (from 32 µg/L at 16 h to 2.8 µg/L at 124 h) (Fig. IV-1&2). In pond T4, in which initial concentration was high, and degradation, slow, survival and the proportion found dead were equally reduced in the shallow and deep zones (Fig. IV-1&2). For *C. tentans*, however, the apparent influence of depth on the toxicity of carbofuran was not clear-cut. Only in pond T1, in which the initial concentration of carbofuran was 9 µg/L, was survival noticeably reduced in the shallow zone when compared with the control (Fig. IV-1). The effect of depth on the toxicity of a given range of concentrations of carbofuran remains speculative. To elucidate its effects, a gradient of depth and insecticide concentrations must be considered while holding constant other, potentially-influential variables (e.g. sediment characteristics).

Regardless of whether depth can influence the toxicity of carbofuran, within-pond variation in the survival of *G. lacustris*, and, to a lesser extent, *C. tentans*, was evident in this study. Such variability may have implications for the survival of mobile benthic invertebrates if they are able to detect and actively avoid potentially lethal concentrations of a pesticide. Detection and avoidance of methoxychlor and fenitrothion have been demonstrated for the stonefly, *Acroneuria lycorias*, the avoidance having been achieved mainly through entry into drift which, in turn, was a consequence of increased activity associated with depressed acetylcholinesterase levels (Scherer & McNicol 1986). These authors also note that, in some cases, invertebrates actually crawled downstream, suggesting that the avoidance reaction may have been, in part, a directed movement as opposed to a mere consequence of non-directed increases in activity.

1987. The toxicity of the 25 µg/L treatment level to *Hyalella* (Table IV-3) agrees with the the post-application declines recorded in the multi-pond (Chapter II) and enclosure studies (Chapter III). However, the significant toxic effect on *Hyalella* at 5 µg/L (Table IV-3) contrasts with the results of the enclosure study (Chapter III), in which no significant decline was recorded. Nevertheless, it was noted in Chapter III that, in the 5-µg/L enclosures, increases in *Hyalella* abundance and biomass were of a slightly smaller magnitude than those in the controls

after carbofuran was applied, possibly as a result of a small effect of carbofuran. Despite the significant differences between the control and 5-µg/L enclosures in the survival and proportion of *Hyalella* found dead, mean differences were slight (Table IV-3), and the ecological significance of this result remains questionable in light of the demonstrated ability of *Hyalella* to recover relatively quickly from insecticide-induced population reductions (Ali & Stanley 1982, this study, Chapter III). The toxic effect of carbofuran, at 25-µg/L, to *C. tentans* (Table IV-3) is consistent with the results of the 1986 cage study as well as the 1987 enclosure study in which a decline in Chironominae biomass was attributed to the susceptibility of large *Chironomus* larvae to the 25-µg/L treatment level (Chapter III). For *C. tentans*, only the proportion of animals found dead was significantly affected by carbofuran at 5 µg/L (Table IV-3). This may have been the result of a toxic effect of carbofuran, a carbofuran-induced decline in the rate of scavenging and/or cannibalism, or some combination of the two. In any event, survival of *C. tentans* was the same in the control and 5-µg/L enclosures and, despite the significant difference, the proportion found dead, was only slightly higher in the 5-µg/L enclosures, suggesting that the effects of carbofuran, at 5 µg/L, were, at best, minimal to this species. This result contrasts with the laboratory-derived LC50 of 1.6 ppb (Karnak & Collins 1974). The toxicity of carbofuran, at 25 µg/L, to *Limnephilus* larvae is consistent with the results of Chapter II, in which a post-treatment decline of trichopteran larvae was recorded. Furthermore, the results suggest that *Limnephilus* larvae would likely be resistant to accidental contaminations of prairie ponds by carbofuran at concentrations of 5 µg/L or less.

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CHAPTER V

THE INFLUENCE OF pH ON MORTALITY RATES OF THE AMPHIPOD, *Hyallela azteca,* EXPOSED TO CARBOFURAN

INTRODUCTION

The toxicities of some carbamate insecticides to various species of fish and macroinvertebrates have been shown to vary with the pH of the water, possibly as a result of the effects of pH on their rates of degradation (Mauck et al. 1977, Fisher & Lohner 1987). Woodward and Mauck (1980) also demonstrated that pH influences the potency of certain carbamates; however, the relationship was complex and did not suggest that this was achieved primarily through its effects on degradation rates.

In a prairie pond of pH 9.0, carbofuran, at 25 µg/L, produced about 80, 90 and 100% mortality of larvae of the midge, *Chironomus tentans,* the amphipod, *Hyalella azteca* (*Hyalella*), and caddisfly larvae of the genus *Limnephilus* respectively; at 5 µg/L, mortality was only about 30% for *Hyalella*, and less than 10% for the other two taxa (Chapter IV). The pH of ponds on the Canadian prairies varies from circumneutral to greater than 9.0 (Wright 1968, Driver & Peden 1977). As with other carbamates, the degradation of carbofuran is accelerated as pH increases above neutrality (Chapman & Cole 1982). However, it is unknown to what extent variation in pH, within a range of values likely to occur in prairie ponds, will affect the potency of carbofuran to aquatic invertebrates. Therefore, the objective of this study was to examine the influence of pH on the potency of carbofuran to *Hyalella,* a common member of the benthos of prairie ponds.

METHODS

Juvenile *Hyalella* were collected in early November, 1987 from a prairie pond described in Chapter III. They were kept in 10-L aquaria containing aerated pond water for 2 weeks before the tests were conducted.

Pond water, which had been passed through a 250-µm sieve to remove all coarse particulate matter, was used in all tests. The

pond water was divided into three separate batches. Each batch was adjusted to pH 7.0, 8.0 or 9.0 using either 0.5 M HCl or 1.0 M NaOH. On the day the test was initiated, glass beakers were filled with 400 mm of the pond water, 25 beakers per pH level. Ten *Hyalella* were added to each beaker and the pH was recorded. Water temperature was 20.5°C in all beakers.

For the test, an emulsifiable formulation (Furadan 480 Flowable®), containing 49.8% carbofuran was used. (HPLC techiques were used to quantify the carbofuran content and the SE estimate was ±1.9%, with n=2). Equal quantities of Furadan and acetone were weighed on a Mettler H10 electronic balance. Carbofuran was then dissolved in the acetone and this solution was diluted in distilled water to yield a stock solution of 40 mg carbofuran/L. Serial dilutions were made from stock to give a range of four test concentrations for each pH level (Table V-1). Each pH level did not receive the same concentrations since preliminary tests had suggested that concentrations yielding mortality in the range of 1-99% would differ among pH levels. The pond water in each beaker received 10 ml of the final solution of a given concentration of carbofuran. Control beakers were treated with 10 ml distilled water containing a quantity of acetone sufficient to yield a concentration of 50 µg acetone/L pond water, a concentration equal to the highest concentration of acetone in the treated beakers. For each pH level, there were five beakers at each of the four concentrations and the control.

After 96 h, the percentage mortality of *Hyalella* was determined in each beaker. Corrections for control mortality were made for each pH level using Abbott's formula (Abbott 1925). Data were probit transformed, and LC50 values, confidence intervals and relative potencies were calculated according to Finney (1947). Slopes of the log concentration-probit mortality lines for the different pH levels, as well as their mean mortalities, adjusted to a common concentration and common slope, were compared using analysis of covariance (Sokal & Rohlf 1981, p.509-530).

RESULTS

Changes in pH were recorded between the beginning and end of the test. At the pH levels designated 7 and 8, mean pH of the water

increased during the 96 h exposure period from 7.0 and 8.0 to about 7.7 and 8.4 respectively, while water which had originally been adjusted to 9.0 declined to about 8.8 (Table V-1). Nevertheless, for the sake of simplicity, I will continue to refer to the different pH levels as 7, 8 and 9. After 96 h, variation within a given pH level remained small (Table V-1) and all levels remained significantly different from one another ($P<0.01$, in all cases) based on Newman-Keuls tests (Snedecor & Cochran 1980, p. 235).

At pH 7, mortality of *Hyalella* was 100% when exposed to carbofuran at 15 µg/L, while, at pH 9 and 12.5 µg carbofuran/L, it was slightly less than control mortality (Table V-2). Thus, these concentrations were not used when calculating LC50 values for their respective pH levels. Estimated LC50's were 8.9, 12.9 and 20.9 µg/L at pH 7, 8 and 9 respectively (Table V-3). Slopes of the concentration-mortality lines did not differ among pH levels ($F=0.10$, 2,44df, $P>0.75$). However, mortality levels, adjusted to a common slope and concentration were significantly different among pH levels ($F=15.34$, 2,46 df, $P<0.001$), indicating that pH influenced the toxicity of carbofuran to *Hyalella*. Carbofuran was estimated to be 1.9-3.0 times more potent at pH 7 than at pH 9, 1.1-2.6 times more potent at pH 8 than 9, and 1.0-1.9 times more potent at pH 7 than 8 (Table V-3). Mean mortality at pH 9, when adjusted to a common slope and concentration (Table V-3), was significantly lower (Newman-Keuls: $P<0.05$) than at the two lower pH levels, while, between pH 7 and 8, differences in adjusted mean mortalities were not significant (Newman-Keuls: $P>0.05$).

DISCUSSION

Because the pH of the water in the test beakers varied somewhat during the test (Table V-1), it would be erroneous to associate the pH levels of 7.0, 8.0 or 9.0 with their respective LC50's for the toxicity of carbofuran to the amphipod, *Hyalella* as determined in this study. Nevertheless, the results indicate that the pH of water, in a range of 7-9, can modify the toxicity of carbofuran to *Hyalella*, with higher pH values being associated with decreased toxicity, possibly as a result of the effect of pH on degradation rates of carbamate insecticides. Estimates of the half-life of carbofuran at pH 7 range from 5.1 (Seiber et al. 1978) to 8.2 weeks (Chapman & Cole 1982), while, at pH 8, a half-life of 1 week was

Table V-1. The pH of water (mean, n=5) in test beakers measured immediately before and after the 96-h test of the toxicity of carbofuran to the amphipod, *Hyalella*.[1]

Concentration (µg/L)	Time	pH 7	pH 8	pH 9
Control	Start	7.0	8.0	9.0
	End	7.7	8.4	8.8
	Avg.	7.4	8.2	8.9
7.5	Start	7.0	8.0	---
	End	7.7	8.4	---
	Avg.	7.4	8.2	---
10.0	Start	7.0	8.0	---
	End	7.7	8.5	---
	Avg.	7.4	8.3	---
12.5	Start	7.0	8.0	9.0
	End	7.7	8.5	8.8
	Avg.	7.4	8.2	8.9
15.0	Start	7.0	8.0	9.0
	End	7.7	8.4	8.8
	Avg.	7.4	8.2	8.9
18.75	Start	---	---	9.0
	End	---	---	8.8
	Avg.	---	---	8.9
25.0	Start	---	---	9.0
	End	---	---	8.8
	Avg.	---	---	8.9

1. Dashed lines indicate that testing was not done at the given combination of pH and carbofuran concentration. Standard errors (±1SE) were <0.05 in all caes.

Table V-2. Percentage mortality (mean±1SE, n=5) of *Hyalella* exposed to various concentrations of carbofuran at three pH levels.[1]

Concentration (µg/L)	pH 7	pH 8	pH 9
0.0	2.0±2.0	10.0±7.7	4.0±2.5
7.5	34.0±7.5	14.0±6.0	---
10.0	64.0±5.1	24.0±4.0	---
12.5	78.0±17.4	36.0±12.9	2.5±2.5
15.0	100.0±0.0	64.0±5.1	12.0±3.8
18.75	---	---	54.0±16.0
25.0	---	---	60.0±7.1

1. Dashed lines indicate that no replicates were tested at a particular combination of pH and carbofuran concentration.

Table V-3. Estimated 96 h LC50's and relative toxicities of carbofuran to *Hyalella* at three pH levels.

pH	96-h LC50 (95% CL)[1] (µg/L)	Adjusted 96-h LC50[2]	Relative Potency[3] pH7	pH8	pH9
7	8.9 (7.36-10.79)	9.0	---	1.0-1.9	1.9-3.0
8	12.9 (11.51-14.42)	12.9	---	---	1.1-2.6
9	20.9 (18.20-23.99)	21.5	---	---	---

1. Concentrations have been backtransformed by taking the antilog$_{10}$ of the log$_{10}$-transformed concentrations.
2. LC50 value adjusted to common slope.
3. 95% confidence limits of difference between means, adjusted to common slope and concentration according to Finney (1947, p.65).

recorded (Chapman & Cole 1982). At pH 8.7, the half-life was further reduced to 19 h (Seiber et al. 1978), and was very similar to the estimated half-life of slightly less than 1 day at pH 9 (NRCC 1979). Hydrolysis of carbofuran to carbofuran phenol was the primary degradative pathway in alkaline rice paddy water (Siddaramappa et al. 1978). Carbofuran phenol was approximately 150-190 times less toxic than carbofuran to rats (NRCC 1979). Thus, it is possible that the more rapid degradation at higher pH levels reduced the duration of exposure of *Hyalella* to the toxic parent compound. These results are consistent with those of Fisher & Lohner (1987), which showed a reduction in the toxicity of carbaryl to larvae of the midge *Chironomus riparius*, as pH increased from 4 to 8, and those of Mauck et al. (1977), which showed that, as the pH increased from 6.5-9.5, the toxicity of mexacarbate to fish increased 38-fold, an increase that, they demonstrated, resulted from the more rapid breakdown of this insecticide at high pH to more toxic compounds. However, these results contrast with those of Woodward and Mauck (1980), which, while demonstrating that pH affected the potencies of two carbamates to fish and aquatic invertebrates, did not suggest that modifications in potency were achieved primarily through the effect of pH on degradation rates.

These results may have implications for the susceptibility of *Hyalella*, and perhaps other aquatic invertebrates, to carbofuran in in prairie ponds. The pH of prairie ponds varies from circumneutral to greater than 9.0 and increases with increasing pond permanency (Wright 1968, Driver & Peden 1977). Therefore, carbofuran contamination within a given range of concentrations may have more severe consequences for populations of a given species of aquatic invertebrate in seasonal ponds of circumneutral pH than for those in permanent ponds of higher pH. Furthermore, if invertebrates in seasonal wetlands of lower pH are more susceptible than those in permanent wetlands of higher pH, waterfowl broods, which depend on aquatic invertebrates as a food source, may also be affected. Seasonal and semi-permanent ponds were preferred wetland types for mallard (*Anas platyrhynchos*) broods in Alberta (Smith 1971), and for all waterfowl broods in N. Dakota (Duebbert & Frank 1984), while Talent et al. (1982) showed that, in years of favorable water conditions, mallards prefer seasonal to semi-permanent wetlands.

Their preference for less permanent, and thus possibly less alkaline waterbodies may be disadvantageous given the higher susceptibility of *Hyalella* (and possibly other invertebrates) to carbofuran at lower pH. Nevertheless, prairie ponds are heterogeneous habitats in many respects, other than pH and degree of permanency (Stewart & Kantrud 1971), and the potency of carbofuran may be modified by other factors such as water temperature, water clarity, sediment characteristics and macrophyte density. Thus, the susceptibility of aquatic invertebrates, such as *Hyalella*, to carbofuran in prairie ponds is likely to be modified, to some extent, not only by pH, but by the interaction of all the environmental variables which affect its bioavailability.

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CHAPTER VI

CONCLUSION

EFFECTS ON MACROINVERTEBRATES.

Based on the results of the multi-pond study, reported in Chapter II, significant declines in the abundance of *Hyalella* and trichopteran larvae could be attributed to the mid-July application of carbofuran in a range of ca. 9-32 µg/L. Trichopteran abundance recovered within one month through recruitment, whereas *Hyalella* abundance remained low into the following spring. Damselfly nymphs and Chironominae larvae were apparently unaffected. In general, secondary effects during the post-spray period were not detected. The results of the within-pond study with enclosures suggested that a concentration of 5 µg/L carbofuran did not decrease significantly the abundance or biomass of seven taxa of macroinvertebrates commonly occurring in prairie ponds. At 25 µg/L, however, *Hyalella* abundance and biomass and Chironominae biomass were reduced to as little as 5-10% of their pre-treatment values. *Hyalella* partially recovered within less than 2 months while Chironominae biomass remained low, possibly because the enclosures prevented colonization by newly-hatched larvae from outside the enclosures. Tanypodinae larvae, snails of the genera *Helisoma* and *Physa* and the leech, *Helobdella stagnalis*, were apparently unaffected by carbofuran at 25 µg/L. The data suggested that ecological release from competition may have resulted in relatively greater numbers and biomass of herbivorous and detritivorous taxa in the 25 µg/L enclosures than in the controls 2 months after treatment. This effect was most noticeable among snails of the genus, *Helisoma*. The container tests (Chapter IV) confirmed the sensitivity of *Hyalella* to carbofuran at 25 µg/L in prairie ponds of pH ca. 9 and also showed a small effect at 5 µg/L. Survival of another amphipod, *G. lacustris*, was also reduced by carbofuran in the range of 9-32 µg/L as was that of *C. tentans* larvae. Caddisfly larvae of the genus *Limnephilus* were sensitive to carbofuran at 25 µg/L but not at 5 µg/L. Survival of damselfly nymphs of the genus *Enallagma* was not significantly reduced in the range of 9-32

µg/L. Within-pond variability in survival of *G. lacustris* and to a lesser extent, *C. tentans*, was evident in three of four ponds, possibly the result of the interaction between water depth and the concentration of carbofuran in a given pond. Survival was enhanced at deep sites in ponds with low initial concentrations of carbofuran and in one pond in which initial concentration was high, but degradation rate was rapid. The results of a laboratory study (Chapter V) showed that the toxicity of carbofuran to *Hyalella* was reduced by Increases in pH above neutrality. These results, in addition to those of other studies suggest that other aquatic invertebrates may be similarly affected. Thus, it is possible that the toxicity of carbofuran to macroinvertebrates in this study may underestimate its toxicity to those in prairie ponds of lower pH. It is noteworthy that the study ponds were at the upper end of a scale of pH values in prairie ponds which have been studied to date.

POTENTIAL EFFECTS ON PRAIRIE-PARKLAND WATERFOWL.

Considering the food webs characteristic of prairie and parkland ponds raises the question: what effects, if any, does the use of carbofuran on the prairies have on ducks? To answer this question it is necessary to consider 1) the likelihood of carbofuran entry into ponds and sloughs and 2) the possible responses of ducks to reduced availability of invertebrates. A final consideration which will not be dealt with here is the direct and secondary toxicities of carbofuran to ducks occupying sloughs at the time they are inadvertently sprayed or feeding on contaminated invertebrates in sloughs shortly after spraying. Direct and secondary toxicities of granular carbofuran to field-feeding birds have been documented (Flickinger et al. 1980, Balcomb 1983, Balcomb et al. 1984) and birds are considered to be highly susceptible to carbofuran poisoning (Hill et al. 1975). Research that is currently being done is focusing on this topic as it relates to mallard ducklings feeding on carbofuran-contaminated foods (P. Martin, Univ. of Guelph, pers. comm.). In the absence of a monitoring program designed to assess the off-target deposition of insecticides for several crops and pests and in several regions of the prairies, the likelihood of carbofuran entry into sloughs remains speculative. That surface runoff into ponds is not likely

to be a potential source of contamination is supported by a study that found that only 0.9-1.9% of applied granular carbofuran was lost in runoff during the growing season (Caro et al. 1976). Although this could conceivably amount to a significant accumulation when runoff from a large field enters a small pond, the rapid degradation of carbofuran in alkaline water would prevent its accumulation over time. Evaluation of the potential for accumulation of carbofuran in prairie ponds via drift deposit and direct overspray remains subjective. Deposits of aerially-applied pesticides within 100 m of the target zone vary widely, from ca. 40-99% of the amount applied (Akesson & Yates 1984), implying that from 1-60% may be deposited beyond 100 m from the target zone. However, deposits at sites ≥ 50 m downwind from the target zone are usually less than ten percent of the amount applied (Sheehan et al. 1987, Table 5-3). Thus, if carbofuran were applied at 140 g/ha, as recommended for the control of grasshoppers, it is arguable that drift deposits into a pond that had an average depth of 1 m and was located 50 m downwind from the spray site , would not exceed 1.4 µg/L. Moreover, since carbofuran is not licensed for use in aquatic habitats, one could argue that the potential for direct overspray is virtually non-existent. On the other hand, the practice of multi-swathing which is common in insect control (Sheehan et al. 1987) may result in much greater accumulation of drift deposits in non-target areas. For example, ten adjacent swaths may result in ca. 200 and 600% increases over one swath in deposits at sites 33 and 167 m respectively downwind from the first swath (Kaupke & Yates 1966). Thus, it is apparent that the practice of multiswathing may result in significant accumulations of carbofuran in waterbodies located downwind from the target field. With respect to the likelihood of direct overflight spraying, Sheehan et al. (1987) have reviewed the attitudes and practices of farmers and aerial applicators on the prairies and have evaluated the practical constraints they face in aerially-spraying farmland with high densities of ponds, and have concluded that partial overspray is highly probable. Furthermore, an unpublished study dealing with the insecticide, fenthion, and cited by Grue et al. (1986), found deposits in excess of 200% of the application rate in wet meadow habitat in Wyoming. Clearly, there is potential for carbofuran contamination of prairie ponds through drift and direct overspray during aerial applications. Whether or not significant

accumulations occur remains unknown.

Given that aerial application of carbofuran may result in its accumulation in some prairie ponds at levels approximating those used in this study, could prairie ducks be adversely affected through the depletion of food resources? To address this question, it is necessary to review what is known of the requirements, food habits and behaviour of species of ducks breeding on the prairies. The importance of high-protein food has been demonstrated for female mallards during egg-laying (Eldridge & Krapu 1988) and for ducklings of several species (Holm & Scott 1954, Johnson 1971, Street 1978) and numerous studies have established the importance of aquatic invertebrates as their main source of high-protein food (e.g. Sugden 1973, Swanson & Meyer 1973,). With some exceptions, ducks appear to feed on most taxa of invertebrates in proportion to their availability in the environment (Bartonek & Hickey 1969a, Bartonek & Murdy 1970, Swanson et al. 1974), suggesting that they are opportunistic feeders, which, when faced with sudden changes in availability of certain prey types, may be capable of switching to other prey as has been demonstrated for generalist predators such as odonates (Akre & Johnson 1979, Wallace et al. 1987). Notwithstanding their potential for switching prey, the consequences of such switches for overall reproductive success and survival remain unknown. Although ducks may switch to less sensitive taxa if sensitive taxa (i.e. *Hyalella, C. tentans*) were decimated in a carbofuran-contaminated pond, a review of their food habits reveals that certain invertebrate taxa, some of which exhibited a high sensitivity to carbofuran in this study, appear to be consistently important in the diets of certain species. Gastropods make up the bulk of the diets of breeding females of several species of ducks while midge larvae are also very important, particularly to ruddy ducks (*Oxyura jamaicensis*), blue-winged teal (*Anas discors*), pintails (*A. acuta*), mallards (*A. platyrhynchos*) and lesser scaup (*Athya affinis*) (Table VI-1). Trichopteran larvae are very common in the diets of breeding canvasbacks (*Athya valisneria*), redheads (*Athya americana*), and ring-necked ducks (*Athya collaris*), while amphipods are consistently important in the diets of breeding lesser scaup (Bartonek & Hickey 1969b, Dirschl 1969, Bartonek & Murdy 1970) (Table VI-1). The high tolerance of

Table VI-1. Composition (mean percentage averaged across studies) of the invertebrate component in the diets of species of prairie ducks during the breeding season.

Taxa	% of total diet by Species										
	Pintail	Mallard	Gadwall	Widgeon	BWT[1]	Shoveler	Canvasback	Redhead	RND[2]	LS[3]	RD[4]
Invertebrates	78	68	72	31	88	99	85	79	78	90	95
Annelida					1				8		
Oligochaeta	10	6									
Hirudinea	1									9	1
Crustacea		7									
Amphipoda			1		5					43	4
Anostraca	12				2	6					
Cladocera			10		8	33					
Other	1		14		2	15			2		
Insecta											
Odonata		5	4	7	1		6	11	3		1
Trichoptera	1	17	4		5		21	42	38	3	1
Coleoptera	3	5	10	3	2	2				1	
Hemiptera			4		1	1					1
Diptera		18									
Chironomidae			22		24	1					
Pupae + Larvae	19						5	7	11	17	63
Adults											
Other	12					7	1			1	

(continued . . .)

Table VI-1. Continued.

Taxa	% of total diet by Species										
	Pintail	Mallard	Gadwall	Widgeon	BWT[1]	Shoveler	Canvasback	Redhead	RND[2]	LS[3]	RD[4]
Mollusca											
Pelycopoda									1	4	
Gastropoda	19	9	2		30	40	46		10	1	22
Other		1	1				7	19	4	13	1
References[5]	e,f	h,o	i,n	a	c,k l,m	n	b,g	b,g	d	b,c	j

1. Blue-winged teal.
2. Ring-necked duck.
3. Lesser scaup.
4. Ruddy duck.
5. a) Bartonek, 1972. b) Bartonek & Hickey 1969b. - females, esophogeal contents only. c) Dirschl 1969. - values used were averages for May and June. d) Hohman 1985. - average values for laying and post-laying females. e) Krapu 1974a. - laying females. f) Krapu 1974b. females feeding on shallow, non-tilled wetlands. g) Noyes & Jarvis 1985. - laying females. h) Perret 1962. i) Serie & Swanson 1976. - laying females. j) Siegfried 1973. - females. k) Swanson 1977. - adults and immatures combined. l). Swanson et al. 1974. females. m) Swanson & Meyer 1977. - laying females. n) Swanson et al. 1979. - laying females. o) Swanson et al. 1985. -laying females.

gastropods to carbofuran in this study suggests that breeding waterfowl would still have access to snails when feeding in a pond contaminated by carbofuran at an earlier date. The importance of snails as a source of protein and calcium for breeding female ducks has been alluded to (Swanson et al. 1979). However, some molluscs contain comparatively low levels of metabolizable energy, (Jorde & Owen 1988); thus, it is unlikely that snails alone would be sufficient to meet all the nutritional and energetic demands of breeding female ducks, particularly those species which do not rely heavily on endogenous energy stores. The sensitivity of large *Chironomus* larvae, the amphipods, *Hyalella* and *G. lacustris,* and trichopteran larvae to carbofuran may have adverse consequences for those species of ducks which depend to a large extent on these taxa. It is noteworthy that large *Chironomus* larvae were the most numerous of the chironomids consumed by blue-winged teal, lesser scaup (Dirschl 1969) and ruddy ducks (Siegfried 1973). Furthermore, since food selection in dabbling ducks (*Anas* spp.) may be positively related to lamellar spacing and body size (Nudds & Bowlby 1984), it is possible that, like the small-bodied blue-winged teal, larger species of dabbling ducks such as mallards and pintails may also prefer the large-bodied *Chironomus* larvae to smaller-bodied chironomids. However, it remains difficult to interpret the potential effect of a carbofuran-induced depletion of *Chironomus* larvae on breeding ducks because, in most cases, prey have only been identified to the level of order or family. Depletion of preferred food resources in a pond might force pairs of ducks which normally use the pond for feeding to seek food on other, uncontaminated ponds which may already be used extensively by other pairs. However, pairs require a certain amount of isolation (Dzubin 1969) and intraspecific crowding may result in behavioural interference and aggression to the point that females will not nest successfully (Titman & Lowther 1975). Krapu et al. (1983) have suggested that habitat deterioration as a result of drought, may concentrate mallards on the remaining wetlands and, this in turn, can lead to increased aggression and abandonment of nesting. It is possible that widespread insecticidal contamination of prairie ponds could act the same way, by forcing pairs onto uncontaminated ponds to feed and, thus, decreasing the amount of isolation they require.

Insecticide-induced reductions of invertebrate abundance and

biomass may be even more harmful to broods than to breeding adults. When the biomass of invertebrates in an insecticide-sprayed pond was reduced by more than 50% when compared with a control pond, the growth rate of captive black duck and mallard ducklings reared on the sprayed pond was reduced and the amount of time they spent searching for food increased relative to ducklings reared on the control pond (Hunter et al. 1984). It has been suggested that decreased resistance to chilling and increased risk of predator attack are possible consequences of lower growth rates and increased time spent searching for food respectively (Sheehan et al. 1987). Moreover, if broods were forced to move to another pond as a result of an insecticide-induced reduction in food, the risk of predation may increase because of the overland movement (Ball et al. 1975). Reductions in abundance and biomass of large species of *Chironomus* (e.g. *C. tentans*) resulting from carbofuran contamination of prairie ponds may be particularly harmful to newborn ducklings of species of dabbling ducks because they rely extensively on emerging chironomids during the first two weeks of life (Chura 1961, Perret 1962, Sugden 1973) and presumably, the larger-bodied species of *Chironomus* would be preferred to smaller species. Moreover, since the hatching of duck eggs begins in late May and continues until mid July across the prairies, interspecific differences in emergence phenologies of chironomids (Wrubleski 1987) may be of crucial importance in providing a continuous source of food for newly-hatched dabbling ducks throughout the summer. The failure of even one species to emerge from a given pond at a particular time may put young ducklings in that pond at risk. Danell and Sjoberg (1977) found that the peak of hatch of ducklings on a northern Swedish lake occurred shortly after the highly synchronous emergence of chironomids and suggested that this hatching schedule may be adaptive. Depletion of other taxa of invertebrates, besides *Chironomus*, may also have adverse effects on ducklings. Lesser scaup ducklings feed extensively on amphipods (Bartonek & Hickey 1969b, Sugden 1973) (Table VI-2); thus, the sensitivity of *Hyallela* and *G. lacustris* to carbofuran may be harmful to ducklings of this species. Trichopteran larvae are important foods of ring-necked duck and canvasback ducklings (Table VI-2); moreover, one study found that this group was important for

Table VI-2. Composition (mean percentage averaged across studies) of the invertebrate component in the diets of ducklings of species of prairie ducks.

Taxa	% Of total diet by Species								
	Pintail	Mallard	Gadwall	Widgeon	Lesser scaup	Canvasback	Redhead	RND[1]	RD[2]
Invertebrates	74	72	10	11	98	88	34	89	94
Annelida									
Hirudinea		1			1				
Crustacea		1							
Amphipoda					51				
Cladocera	2		2		1		4		
Other	4							5	
Insecta									
Odonata	2	2		1	2	6	2	1	
Trichoptera	1	12		1	1	43	9	53	3
Coleoptera	3	6	3						1
Hemiptera	3	4		1	3		5		
Diptera		35							
Chironomidae	13								
Pupae + Larvae			2		11	7	4	24	73
Adults			2	4					
Other	2			1	1				

(continued . . .)

Table VI-2. Continued.

Taxa	% of total diet by Species								
	Pintail	Mallard	Gadwall	Widgeon	Lesser scaup	Canvasback	Redhead	RND[1]	RD[2]
Mollusca Gastropoda	42	6		2	27	30	7	4	
Other	2	3				2	3	2	
References[3]	e,h	b,f	h	h	a,h	a,d	a,d	c	g

1. Ringed-neck duck.
2. Ruddy duck.
3. a) Bartonek & Hickey 1969b. - values for esophogeal contents. b) Chura 1961. - Classes I & II combined and averaged. c) Hohman 1985. - classes I & II combined and averaged. d) Jarvis & Noyes 1986. - classes I & II combined and averaged. e) Krapu & Swanson 1977. - values for flightless ducklings. f) Perret 1962. g) Siegfried 1973. h) Sugden 1973. - values from tables 13 & 14 (dry wt.).

redhead ducklings (Bartonek & Hickey 1969b) while another found that they were important for mallard ducklings (Perret 1962). Presumably, the sensitivity of trichopteran larvae to carbofuran may have harmful effects on these species, particularly if it is sprayed earlier in the year when most of the trichopteran species have not yet emerged and begun to breed and lay eggs as may have occurred in the multi-pond study (Chapter II).

Clearly, the extent to which breeding waterfowl and their broods would be adversely affected by insecticide-induced reductions in invertebrates depends largely on the magnitude of the reduction in key invertebrate groups such as chironomids, trichopterans and amphipods, as well as on the proximity of suitable alternate feeding locations, which, in turn, may be affected by the extensiveness of the spray program. The results of this study suggest that in ponds of pH 9, carbofuran at 25 µg/L (or perhaps somewhat lower) may reduce certain invertebrate taxa sufficiently to adversely affect some species of ducks, especially mallards, lesser scaup, canvasbacks, ring-necked ducks, ruddy ducks and, possibly, redheads, which rely heavily on one or more of the sensitive invertebrate taxa. In ponds of lower pH, harmful effects might be evident at lower concentrations. Potential effects of invertebrate reduction on zooplankton feeding species such as the northern shoveler (*A. clypeata*), and, to a lesser extent, the blue-winged teal, remain to be evaluated.

Finally, the potential hazard to macroinvertebrates of carbofuran contamination of a theoretical prairie pond of certain dimensions was estimated to be quite low (Sheehan et al. 1987). Their hazard assessment was based on an initial carbofuran concentration of 17 µg/L. The data presented in this thesis suggest that their assessment may have been overly optimistic. It appears that, at 17 µg/L, carbofuran may be acutely toxic to at least some taxa of macroinvertebrates known for their importance as foods for some species of prairie ducks.

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APPENDIX I. Relative abundance[1] of the various macroinvertebrate taxa collected during the 1986 multi-pond study in each of the study ponds.

Taxon	Pond C1	C2	C3	C4	T1	T2	T3	T4
Annelida								
Oligochaeta								
Hirudinea								
Glossiphoniidae								
Helobdella stagnalis	+	+	+	+	+	+	+	+
H.triserialis	0	0	0	+	+	0	0	0
H. elongata	0	0	0	0	+	0	0	0
Theromyzon	0	0	0	0	0	0	+	0
Placobdella	0	+	+	0	+	0	0	0
Glossiphonia complanata	+	+	+	+	+	+	+	+
Alboglossiphonia heteroclita	0	0	0	0	+	0	+	0
Erpobdellidae								
Nephelopsis obscura	+	+	+	+	+	+	+	+
Erpobdella punctata	+	+	+	+	+	+	+	+

APPENDIX I. Continued.

Taxon	Pond							
	C1	C2	C3	C4	T1	T2	T3	T4
Mollusca								
Gastropoda								
Physidae								
Physa	+	++	+	++	+	+	+	+
Lymnaeidae								
Stagnicola	+	+	0	0	0	+	0	+
Lymnaea	+	+	0	0	+	+	0	+
Planorbidae								
Gyraulus	0	+	0	0	0	+	+	+
Armiger	0	0	0	0	0	0	0	+
Helisoma	+	+	0	0	0	0	+	0
Pelecyopoda								
Sphaeriidae								
Pisidium	++	+	++	+++	++	+	++	+
Arthropoda								
Crustacea								
Amphipoda								
Gammaridae								
Gammarus lacustris	++	+++	+	++	++	++	++	+

APPENDIX I. Continued..

Taxon	Pond							
	C1	C2	C3	C4	T1	T2	T3	T4
Talitridae								
Hyalella azteca	++	+++	++	++	++	+	++	++
Acarina								
Hydracarina								
Arrenuridae								
Arrenurus	0	+	0	+	+	+	0	0
Eylaidae								
Eylais	0	0	0	0	+	0	0	0
Hydrachnidae								
Hydrachna	0	+	0	+	0	+	+	0
Hydrodromidae								
Hydrodroma	+	0	0	+	0	0	0	0
Limnesidae								
Limnesia	+	+	+	+	+	+	+	+
Limnocharidae								
Limnochares	+	0	+	0	0	0	0	0
Pionidae	0	0	+	0	+	0	0	0

APPENDIX I. Continued.

Taxon	Pond							
	C1	C2	C3	C4	T1	T2	T3	T4
Insecta								
Ephemeroptera								
Caenidae								
Caenis	++	++	++	++	++	++	++	++
Baetidae								
Callibaetis	+	++	++	+	+	++	+	+
Cloeon	0	0	+	0	0	0	+	0
Anisoptera								
Libellulidae								
Libellula	+	+	0	+	+	+	+	+
Sympetrum	0	0	+	0	0	0	+	+
Aeshnidae								
Aeshna	0	+	0	0	+	+	0	0
Zygoptera								
Coenagrionidae	+	++	+	++	+	++	++	++
Lestidae								
Lestes	0	+	0	0	+	+	0	+
Hemiptera								
Corixidae	+	+	+	+	+	+	+	+

APPENDIX I. Continued.

Taxon	Pond							
	C1	C2	C3	C4	T1	T2	T3	T4
Notonectidae								
Buenoa	0	+	0	0	0	+	0	+
Notonecta	0	+	+	0	0	+	0	+
Trichoptera								
Leptoceridae								
Triaenodes	0	+	+	+	0	+	+	0
Mystacides	+	+	+	+	0	0	+	0
Oecetis	+	+	+	0	0	+	+	0
Limnephilidae								
Anabolia	0	+	0	+	0	0	+	+
Limnephilus	+	+	+	+	+	++	+	+
Nemotaulius	+	+	0	0	0	0	+	+
Molannidae								
Molanna	+	+	+	+	+	+	+	+
Phryganeidae								
Agrypnia	+	+	+	+	+	+	0	+
Polycentropodidae								
Polycentropus	0	+	0	+	+	+	+	+
Coleoptera								
Dytiscidae								
Colymbetes	0	+	0	0	+	+	+	+

APPENDIX I. Continued.

Taxon	Pond							
	C1	C2	C3	C4	T1	T2	T3	T4
Dytiscus	+	+	+	0	+	0	0	+
Graphoderus	+	+	0	0	0	0	0	+
Hydroporus	+	+	+	+	+	+	+	+
Hygrotus	+	+	0	+	+	0	0	+
Ilybius	+	+	0	+	+	+	+	+
Laccophilus	+	+	+	+	+	+	+	+
Hydrophilidae								
Helophorus	0	+	0	0	+	+	0	+
Hydrochus	0	0	0	0	+	0	0	+
Enorchus	0	+	0	0	0	+	0	0
Elmidae								
Dubiraphia	0	+	0	+	0	+	+	0
Chrysomelidae								
Donacia	+	+	0	+	+	+	+	0
Diptera								
Nematocera								
Ceratopogonidae	+	+	0	+	+	+	+	+
Chaoboridae								
Chaoborus	++	+	++	0	++	++	+	+
Chironomidae								
Chironominae	++	+++	+++	+++	+++	+	++	++

APPENDIX I. Continued.

Taxon	Pond							
	C1	C2	C3	C4	T1	T2	T3	T4
Tanypodinae	++	+++	++	++	++	++	++	++
Orthocladinae	++	++	+	+	++	+	+	++
Dixidae								
Dixella	0	0	0	0	+	0	0	+
Tipulidae								
Tipula	0	0	0	0	0	0	+	+
Brachycera								
Tabanidae								
Chrysops	+	+	+	+	+	+	+	+
Tabanus	+	0	0	0	+	0	0	0
Cyclorrapha								
Anthomyiidae	0	0	0	0	0	0	0	+
Ephydridae	0	+	0	0	0	0	0	+

1. +++ - abundant (≥10 individuals per sample in ≥50% of the samples from the indicated pond).
   ++ - common (≥1 individual per sample in ≥50% of the samples from the indicated pond).
   + - rare (occuring in <50% of the samples taken from the indicated pond).
   0 - absent in all samples taken from the indicated pond.

APPENDIX II. Relative abundance[1] of macroinvertebrates found in samples taken from the 21 enclosures in the study pond during summer, 1987.

Taxon	Control	5 µg/L	25 µg/L
Annelida			
Hirudinea			
Glossiphoniidae			
Glossiphonia complanata	+	+	+
Helobdella stagnalis	++	++	++
H. elongata	+	+	+
H. triserialis	+	+	+
Theromyzon	+	+	+
Erpobdellidae			
Erpobdella punctata	+	+	+
Nephelopsis obscura	+	+	+
Unknown	+	+	+
Mollusca			
Gastropoda			
Physiidae			
Physa	++	++	++
Lymnaeidae			
Lymnaea	+	0	0
Planorbidae			
Helisoma	++	++	++
Menetus	+	+	+
Arthropoda			
Crustacea			
Amphipoda			
Gammaridae			
Gammarus lacustris	+	0	0
Talitridae			
Hyalella azteca	++++	++++	+++
Arachnida			
Acarina			
Hyrdacarina			
Arrenuridae			
Arrenurus	+	0	+
Hydrodromidae			
Hydrodroma	+	0	0
Limnesiidae			
Limnesia	+	+	+
Eylaidae			
Eylais	+	+	0

APPENDIX II. Continued.

Taxon	Control	5 µg/L	25 µg/L
Insecta			
Anisoptera			
Libellulidae			
Libellula	+	0	+
Leucorrhinia	0	+	+
Zygoptera			
Coenagrionidae	++	++	++
Lestidae			
Lestes	+	+	+
Ephemeroptera			
Caenidae			
Caenis	++	++	++
Trichoptera			
Phryganeidae			
Phryganea	+	+	0
Agrypnia	0	+	0
Limnephilidae			
Limnephilus	+	+	0
Leptoceridae			
Triaenodes	0	+	0
Hemiptera			
Corixidae - Nymph	+	+	+
- Adult	+	+	+
- Both	++	+	++
Notonectidae - Nymph	+	+	+
- Adult	+	+	+
- Both	+	+	+
Coleoptera			
Dytiscidae			
Hydroporus - Adult	+	0	0
Hygrotus - Larvae	+	+	+
- Adult	+	0	+
- Both	+	+	+
Laccophilus - Larvae	+	0	0
Ilybius - Larvae	+	+	+
Colymbetes - Adult	+	+	+
Rhantus - Larvae	+	+	+
- Adult	+	0	0
- Both	+	+	+

APPENDIX II. Continued.

Taxon	Control	5 µg/L	25 µg/L
Diptera			
Chironomidae			
Chironominae	+++	+++	+++
Chironomus	++	++	+
Tanypodinae	+++	+++	+++
Orthocladinae	++	++	++
Chaoboridae			
Chaoborus	++	++	++
Ceratopogonidae	+	+	+

1. ++++ - very abundant. ≥100 individuals per sample in ≥50% of the samples.
   +++ - abundant. ≥10 individuals per sample in ≥50%of the samples.
   ++ - common. ≥1 individual per sample in ≥50% of the samples.
   + - rare. Occuring in less than 50% of the samples.
   0 - absent in all samples at the indicated treatment level.

APPENDIX III. Coefficient of variation (%) of the four taxa which were common to both the 1986 multi-pond and 1987 enclosure studies.

Taxon	Multi-Pond (n=40)		Enclosure (n=126)	
	Residual[1]	Pond[2]	Residual[1]	Pond[2]
Hyalella	34[3]	80	18	19
	49[4]	118		
Caenis	40	48	53	75
	47[4]	58		
Chironominae	25	63	35	53
Coenagrionidae	21	90	46	77

1. CV=100 x SQRT(Variance due to DATE X POND or ENCLOSURE within TREATMENT) ÷ overall mean
2. CV=100 x SQRT(Variance due to POND or ENCLOSURE within TREATMENT) ÷ overall mean
3. Based on analysis from shallow zones of ponds.
4. Based on analysis from deep zones of ponds.

NOTE: SQRT=square root

APPENDIX IV. Comparisons of means of different dates within a treatment level.[1]

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Hyalella-Ab.[2] (No./sample)	July 8	Control	58.0	0.99ns	--	--	--	--
		5 µg/L	65.1	1.26ns	--	--	--	--
		25 µg/L	118.6	0.24ns	--	--	--	--
	July 14	Control		90.0	1.52ns	--	--	--
		5 µg/L		110.2	0.70ns	--	--	--
		25 µg/L		130.5	4.74**	--	--	--
	July 20	Control			158.8	0.62ns	1.52ns	1.52ns
		5 µg/L			143.3	0.82ns	2.22ns	1.45ns
		25 µg/L			12.8	0.02ns	1.85ns	4.39**
	July 26	Control				197.8	2.63ns	0.90ns
		5 µg/L				191.5	1.40ns	0.64ns
		25 µg/L				12.2	1.92ns	4.46**
	Aug 16	Control					440.0	1.73ns
		5 µg/L					302.4	0.75ns
		25 µg/L					37.2	2.54ns
	Sept 8	Control						277.1
		5 µg/L						238.5
		25 µg/L						114.5

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Hyalella-Bm.[3] (mg/sample)	July 8	Control	50.8	1.08ns	--	--	--	--
		5 µg/L	63.2	1.89ns	--	--	--	--
		25 µg/L	224.3	0.24ns	--	--	--	--
	July 14	Control		98.5	2.28ns	--	--	--
		5 µg/L		175.6	0.69ns	--	--	--
		25 µg/L		181.4	4.41**	--	--	--
	July 20	Control			293.8	0.10ns	1.01ns	0.42ns
		5 µg/L			241.0	0.63ns	0.74ns	0.10ns
		25 µg/L			9.0	0.24ns	1.00ns	3.82**
	July 26	Control				282.6	1.12ns	0.93ns
		5 µg/L				181.4	1.37ns	0.75ns
		25 µg/L				11.2	1.24ns	3.57**
	Aug 16	Control					443.9	0.62ns
		5 µg/L					332.4	0.62ns
		25 µg/L					32.6	2.32ns
	Sept 8	Control						351.5
		5 µg/L						256.0
		25 µg/L						135.2

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Chironominae-Ab.[2] (No./sample)	July 8	Control	40.2	0.10ns	--	--	--	--
		5 µg/L	19.3	1.39ns	--	--	--	--
		25 µg/L	25.2	2.16ns	--	--	--	--
	July 14	Control		41.5	1.89ns	--	--	--
		5 µg/L		30.7	1.04ns	--	--	--
		25 µg/L		22.6	2.37ns	--	--	--
	July 20	Control			23.6	0.79ns	0.79ns	1.20ns
		5 µg/L			21.8	0.35ns	0.54ns	2.80*
		25 µg/L			7.7	1.48ns	0.10ns	2.26ns
	July 26	Control				30.5	1.58ns	1.99ns
		5 µg/L				19.3	0.20ns	2.46ns
		25 µg/L				15.7	1.45ns	0.84ns
	Aug 16	Control					17.7	0.41ns
		5 µg/L					17.8	2.26ns
		25 µg/L					7.6	2.28ns
	Sept 8	Control						15.0
		5 µg/L						5.5
		25 µg/L						21.7

APPENDIX IV. Continued.

Variable	Date	Treatment	DATE July 8	July 14	July 20	July 26	Aug 16	Sept 8
Chironominae-Bm.[3] (mg/sample)	July 8	Control	31.6	1.66ns	--	--	--	--
		5 µg/L	26.9	1.63ns	--	--	--	--
		25 µg/L	13.8	2.16ns	--	--	--	--
	July 14	Control		74.1	0.89ns	--	--	--
		5 µg/L		61.7	0.59ns	--	--	--
		25 µg/L		40.7	4.20**	--	--	--
	July 20	Control			46.8	0.96ns	3.68**	4.08**
		5 µg/L			45.7	1.89ns	4.16**	5.80**
		25 µg/L			4.9	0.78ns	1.74ns	0.20ns
	July 26	Control				28.8	2.72ns	3.12*
		5 µg/L				17.4	2.27ns	3.91**
		25 µg/L				7.2	2.52ns	0.97ns
	Aug 16	Control					7.4	0.40ns
		5 µg/L					5.5	1.54ns
		25 µg/L					2.0	1.54ns
	Sept 8	Control						6.0
		5 µg/L						2.4
		25 µg/L						4.5

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Tanypodinae-Ab.[2] (No./sample)	July 8	Control	59.7	0.08ns	--	--	--	--
		5 µg/L	27.0	0.30ns	--	--	--	--
		25 µg/L	45.7	0.54ns	--	--	--	--
	July 14	Control		60.6	2.18ns	--	--	--
		5 µg/L		24.7	0.45ns	--	--	--
		25 µg/L		39.1	0.71ns	--	--	--
	July 20	Control			33.7	1.33ns	0.83ns	1.60ns
		5 µg/L			27.5	0.99ns	1.30ns	3.54**
		25 µg/L			32.1	0.92ns	0.71ns	0.57ns
	July 26	Control				48.6	2.16ns	2.94*
		5 µg/L				37.2	2.20ns	4.53**
		25 µg/L				41.6	1.63ns	0.35ns
	Aug 16	Control					26.1	0.77ns
		5 µg/L					18.7	2.33ns
		25 µg/L					25.6	1.28ns
	Sept 8	Control						20.2
		5 µg/L						7.8
		25 µg/L						37.8

APPENDIX IV. Continued.

Variable	Date	Treatment	Date July 8	July 14	July 20	July 26	Aug 16	Sept 8
Tanypodinae-Bm.[3] (mg/sample)	July 8	Control	24.5	1.98ns	--	--	--	--
		5 µg/L	25.7	0.73ns	--	--	--	--
		25 µg/L	12.9	1.29ns	--	--	--	--
	July 14	Control		69.2	0.10ns	--	--	--
		5 µg/L		37.2	0.45ns	--	--	--
		25 µg/L		25.7	0.92ns	--	--	--
	July 20	Control			66.1	0.49ns	1.49ns	2.19ns
		5 µg/L			29.5	0.82ns	0.24ns	3.21**
		25 µg/L			15.8	1.76ns	0.61ns	0.65ns
	July 26	Control				50.1	1.00ns	1.70ns
		5 µg/L				19.1	1.06ns	2.39ns
		25 µg/L				39.8	1.15ns	1.10ns
	Aug 16	Control					29.5	0.70ns
		5 µg/L					33.9	3.45**
		25 µg/L					21.9	0.04ns
	Sept 8	Control						20.4
		5 µg/L						5.4
		25 µg/L						22.4

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Physa-Ab.[2] (No./sample)	July 8	Control	2.8	1.58ns	--	--	--	--
		5 µg/L	3.5	0.65ns	--	--	--	--
		25 µg/L	2.7	1.81ns	--	--	--	--
	July 14	Control		5.1	1.24ns	--	--	--
		5 µg/L		2.8	0.09ns	--	--	--
		25 µg/L		5.5	2.04ns	--	--	--
	July 20	Control			3.1	0.30ns	4.44**	4.35**
		5 µg/L			2.9	0.78ns	3.63**	2.22ns
		25 µg/L			2.5	1.63ns	3.61**	5.91**
	July 26	Control				2.8	4.81**	4.64**
		5 µg/L				3.9	2.85ns	1.44ns
		25 µg/L				4.7	1.98ns	4.27**
	Aug 16	Control					18.2	0.17ns
		5 µg/L					11.7	1.41ns
		25 µg/L					10.0	2.29ns
	Sept 8	Control						17.0
		5 µg/L						6.8
		25 µg/L						24.5

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Physa-Bm.[3] (mg/sample)	July 8	Control	3.2	2.91*	--	--	--	--
		5 µg/L	4.8	0.05ns	--	--	--	--
		25 µg/L	4.9	1.59ns	--	--	--	--
	July 14	Control		17.4	2.09ns	--	--	--
		5 µg/L		5.0	0.05ns	--	--	--
		25 µg/L		12.3	1.84ns	--	--	--
	July 20	Control			5.1	0.34ns	4.51**	3.75**
		5 µg/L			4.8	0.94ns	2.44ns	4.70**
		25 µg/L			4.2	1.31ns	1.89ns	1.30ns
	July 26	Control				4.2	4.77**	4.09**
		5 µg/L				8.3	0.95ns	3.36**
		25 µg/L				9.1	1.13ns	3.39**
	Aug 16	Control					67.6	0.68ns
		5 µg/L					14.5	0.60ns
		25 µg/L					17.4	2.25ns
	Sept 8	Control						45.7
		5 µg/L						10.2
		25 µg/L						64.6

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Helisoma-Ab.[2] (No./sample)	July 8	Control	0.2	2.30ns	--	--	--	--
		5 µg/L	0.5	0.79ns	--	--	--	--
		25 µg/L	0.8	0.98ns	--	--	--	--
	July 14	Control		2.6	0.96ns	--	--	--
		5 µg/L		1.2	0.44ns	--	--	--
		25 µg/L		2.1	0.11ns	--	--	--
	July 20	Control			1.0	1.18ns	0.82ns	2.00ns
		5 µg/L			1.9	0.20ns	0.10ns	1.94ns
		25 µg/L			2.4	1.05ns	1.13ns	0.03ns
	July 26	Control				3.1	0.36ns	3.18**
		5 µg/L				2.4	0.30ns	2.14ns
		25 µg/L				5.6	0.08ns	1.02ns
	Aug 16	Control					2.3	2.82*
		5 µg/L					1.7	1.84ns
		25 µg/L					6.1	1.10ns
	Sept 8	Control						0.1
		5 µg/L						0.2
		25 µg/L						2.8

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Helisoma-Bm.[3] (mg/sample)	July 8	Control	5.9	1.87ns	--	--	--	--
		5 µg/L	4.5	1.99ns	--	--	--	--
		25 µg/L	14.1	0.40ns	--	--	--	--
	July 14	Control		36.3	0.23ns	--	--	--
		5 µg/L		30.9	0.03ns	--	--	--
		25 µg/L		20.9	0.35ns	--	--	--
	July 20	Control			28.8	0.46ns	0.65ns	1.91ns
		5 µg/L			31.6	0.67ns	0.72ns	0.81ns
		25 µg/L			28.8	1.60ns	2.19ns	1.56ns
	July 26	Control				45.7	0.19ns	2.37ns
		5 µg/L				60.2	0.05ns	1.48ns
		25 µg/L				138.0	0.59ns	0.04ns
	Aug 16	Control					55.0	2.56ns
		5 µg/L					64.6	1.53ns
		25 µg/L					239.9	0.63ns
	Sept 8	Control						4.6
		5 µg/L						14.5
		25 µg/L						131.8

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Coenagrionidae-Ab[2] (No./sample)	July 8	Control	1.1	3.42**	--	--	--	--
		5 µg/L	1.3	1.88ns	--	--	--	--
		25 µg/L	1.1	2.14ns	--	--	--	--
	July 14	Control		4.1	0.56ns	--	--	--
		5 µg/L		2.7	0.58ns	--	--	--
		25 µg/L		2.5	1.17ns	--	--	--
	July 20	Control			5.1	0.41ns	2.02ns	2.29ns
		5 µg/L			3.3	2.09ns	2.88*	3.49**
		25 µg/L			3.9	1.63ns	1.06ns	3.82**
	July 26	Control				6.0	1.61ns	1.81ns
		5 µg/L				7.4	0.79ns	1.40ns
		25 µg/L				7.4	0.57ns	2.19ns
	Aug 16	Control					11.1	0.27ns
		5 µg/L					10.0	0.61ns
		25 µg/L					5.9	2.76ns
	Sept 8	Control						12.3
		5 µg/L						12.7
		25 µg/L						17.1

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Coenagrionidae-Bm[3] (mg/sample)	July 8	Control	1.0	2.29ns	--	--	--	--
		5 µg/L	1.1	1.58ns	--	--	--	--
		25 µg/L	1.2	1.06ns	--	--	--	--
	July 14	Control		2.4	0.23ns	--	--	--
		5 µg/L		2.0	1.68ns	--	--	--
		25 µg/L		1.8	0.14ns	--	--	--
	July 20	Control			2.4	0.23ns	2.94*	5.55**
		5 µg/L			1.4	1.68ns	4.78**	5.70**
		25 µg/L			1.9	1.17ns	2.15ns	5.60**
	July 26	Control				2.6	2.71ns	5.32**
		5 µg/L				2.7	3.10**	4.02**
		25 µg/L				3.0	0.98ns	4.43**
	Aug 16	Control					7.4	2.61ns
		5 µg/L					8.9	0.92ns
		25 µg/L					4.3	3.45**
	Sept 8	Control						19.9
		5 µg/L						12.7
		25 µg/L						16.2

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Caenis-Ab.[2] (No./sample)	July 8	Control	1.1	0.72ns	--	--	--	--
		5 µg/L	1.5	0.36ns	--	--	--	--
		25 µg/L	1.1	0.35ns	--	--	--	--
	July 14	Control		1.6	0.11ns	--	--	--
		5 µg/L		1.3	0.03ns	--	--	--
		25 µg/L		1.3	0.12ns	--	--	--
	July 20	Control			1.5	1.46ns	6.12**	5.14**
		5 µg/L			1.3	0.70ns	4.46**	4.87**
		25 µg/L			1.2	2.38ns	6.54**	5.81**
	July 26	Control				3.2	4.66**	3.68**
		5 µg/L				1.8	3.76**	4.17**
		25 µg/L				4.4	4.16**	3.43**
	Aug 16	Control					37.6	0.98ns
		5 µg/L					13.6	0.41ns
		25 µg/L					40.1	0.73ns
	Sept 8	Control						22.3
		5 µg/L						16.9
		25 µg/L						27.2

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
Caenis-Bm.[3] (mg/sample)	July 8	Control	1.1	0.72ns	--	--	--	--
		5 µg/L	1.5	0.36ns	--	--	--	--
		25 µg/L	1.1	0.35ns	--	--	--	--
	July 14	Control		1.6	0.11ns	--	--	--
		5 µg/L		1.3	0.03ns	--	--	--
		25 µg/L		1.3	0.12ns	--	--	--
	July 20	Control			1.5	1.46ns	6.12**	5.14**
		5 µg/L			1.3	0.70ns	4.46**	4.87**
		25 µg/L			1.2	2.38ns	6.54**	5.81**
	July 26	Control				3.2	4.66**	3.68**
		5 µg/L				1.8	3.76**	4.17**
		25µg/L				4.4	4.16**	3.43**
	Aug 16	Control					37.6	0.98ns
		5 µg/L					13.6	0.41ns
		25 µg/L					40.1	0.73ns
	Sept 8	Control						22.3
		5 µg/L						16.9
		25 µg/L						27.2

APPENDIX IV. Continued.

Variable	Date	Treatment	Date: July 8	July 14	July 20	July 26	Aug 16	Sept 8
H. stagnalis-Ab.[2] (No./sample)	July 8	Control	3.5	0.19ns	--	--	--	--
		5 µg/L	2.8	0.84ns	--	--	--	--
		25 µg/L	3.8	1.07ns	--	--	--	--
	July 14	Control		3.3	0.48ns	--	--	--
		5 µg/L		3.6	1.33ns	--	--	--
		25 µg/L		2.6	0.26ns	--	--	--
	July 20	Control			2.8	0.38ns	3.35**	1.82ns
		5 µg/L			5.8	0.55ns	0.86ns	0.21ns
		25 µg/L			2.7	0.14ns	1.97ns	1.12ns
	July 26	Control				3.2	2.97*	1.44ns
		5 µg/L				4.8	1.41ns	0.76ns
		25 µg/L				3.4	1.83ns	0.98ns
	Aug 16	Control					8.9	1.53ns
		5 µg/L					7.8	0.65ns
		25 µg/L					4.8	0.85ns
	Sept 8	Control						5.2
		5 µg/L						6.2
		25 µg/L						3.5

APPENDIX IV. Continued.

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1. Values along diagonal are means, backtransformed from square-root (SQRT), fourth-root (4THRT), or logarithmic (LOG10) transformations of original data. Other values are adjusted t-statistics using 90df and 8 comparisons (Rohlf & Sokal 1981, p.83) .
   ns - not significant (P>.05).
   * - (.01<P<.05).
   ** - (P<.01).
2. Ab.=abundance.
3. Bm.=biomass (dry weight).

LITERATURE CITED

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